THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

NORTH OF ENGLAND AND SCOTTISH SECTIONS

A JOINT Meeting of the North of England and Scottish Sections was held at 7.30 p.m. on Friday, April 28th, 1961, in the Central Hotel, Victoria Viaduct, Carlisle. The Chair was taken by the Chairman of the North of England Section, Mr. J. Markland, B.Sc., F.R.I.C.

The following papers were presented and discussed: "The Determination of Nitrates and the Application of 'Dead-stop' Titrimetry," by A. F. Williams, B.Sc., F.R.I.C.; "Ion-exchange Resins as Analytical Tools," by T. R. E. Kressman, B.Sc., Ph.D., F.R.I.C.

The meeting was preceded in the afternoon by a visit to the U.K.A.E.A. Power Station at Chapelcross.

WESTERN SECTION

A JOINT Meeting of the Western Section with the Cardiff and District Section of the Royal Institute of Chemistry, the South Wales Section and the Food Group of the Society of Chemical Industry was held at 7 p.m. on Friday, April 28th, 1961, at University College, Cardiff. The Chair was taken by Mr. S. Dixon, M.Sc., F.R.I.C.

A lecture on "Modern Legislation in Relation to Food Additives" was given by C. A. Adams, C.B.E., B.Sc., F.R.I.C., Barrister-at-Law.

The meeting was preceded in the afternoon by a visit, by courtesy of Messrs. P. Leiner (Wales) Ltd., to their Treforest works.

MIDLANDS SECTION AND PHYSICAL METHODS GROUP

A JOINT Meeting of the Midlands Section and the Physical Methods Group was held at 7 p.m. on Wednesday, April 26th, 1961, in the Main Chemistry Lecture Theatre, The University, Edgbaston, Birmingham, 15. The Chair was taken by the Chairman of the Midlands Section, Dr. H. C. Smith, M.Sc., F.R.I.C., Dip.Ed.

The following papers were presented and discussed: "Spectrofluorimetry," by C. A. Parker, B.Sc., Ph.D., F.R.I.C.; "Tesla-luminescence Spectra," by R. J. Magee, M.Sc., Ph.D., A.R.I.C., M.R.S.H. (see summaries below).

The meeting was preceded at 3 p.m. by a visit to the Mond Nickel Co. Ltd., Wiggin Street, Birmingham, 16.

SPECTROFLUORIMETRY

DR. C. A. PARKER recalled that the visible fluorescence of solutions of certain compounds when exposed to ultra-violet light had been used for many years for the determination of such organic materials as vitamins and condensed-ring aromatic hydrocarbons. It had also been used to detect traces of metals that could be made to react with a reagent to give a fluorescent compound. The technique could be made much more specific and sensitive by using a modern photomultiplier tube and a spectrometer to analyse the spectrum of the fluorescent light or to isolate specific frequencies of light for excitation of fluorescence. If an ultra-violet detector was used, the method was applicable to a wide range of substances, because many aromatic compounds fluoresced in the ultra-violet region of the spectrum.

The range of frequencies at which the band of fluorescent light appeared was directly related to the energy differences between the ground state and the first excited state of the molecule and provided one criterion for identification. The shape of the fluorescence band was generally independent of the frequency of the exciting light, but a second

criterion for identification could be obtained by plotting its intensity as a function of the frequency of the exciting light (the excitation spectrum). Spectrofluorimetry thus had two advantages over absorption spectrophotometry. First, it provided two characteristic spectra instead of one, and second, it was capable of measuring much lower concentrations. It was somewhat less widely applicable than absorption spectrophotometry, because not every absorbing substance fluoresced—at least at room temperature.

The ideal general-purpose spectrofluorimeter required the use of two wide-aperture spectrometers and was expensive. However, very useful work could be done with a simpler arrangement in which only one spectrometer was used (*e.g.*, the monochromator from a commercial absorption spectrophotometer).

The technique had been much employed in recent years, particularly in the United States of America, for the determination of low concentrations of compounds of biochemical significance. It could also be used in inorganic trace analysis, and the author described some applications from his own experience.

Tesla-luminescence Spectra

Dr. R. J. Magee said that the phenomenon of tesla-luminescence had first been investigated some thirty years ago by workers in the Chemistry Department of The Queen's University, Belfast. After a few years, however, for reasons unknown, interest in the subject had died out. Recently, with modern methods and a new approach, the field had been re-investigated. This paper gave a report on the progress of these re-investigations. The new developments included a simplified tesla-generator, sample tubes and improved methods for the detection of the spectra obtained. The author gave details of the equipment used and presented the results obtained with a number of organic and inorganic compounds. He discussed the range and possibilities of the technique.

MIDLANDS SECTION and MICROCHEMISTRY GROUP

A Joint Meeting of the Midlands Section and the Microchemistry Group was held at 7.15 p.m. on Friday, May 12th, 1961, in the Nottingham and District Technical College, Burton Street, Nottingham. The Chair was taken by the Chairman of the Microchemistry Group, Mr. C. Whalley, B.Sc., F.R.I.C.

The subject of the meeting was "Automation in the Analytical Laboratory" and the following papers were presented and discussed: "The Scope of Automation in the Laboratory," by G. Mattock, B.Sc., Ph.D., A.R.I.C. (see summary below); "A Colorimeter-type Instrument for the Continuous and Automatic Analysis of Gases in the Parts per Million Range" and "An Automatic Titrimeter," by M. Akhtar, Ph.D., M.Sc., D.I.C.

The meeting was preceded at 2.30 p.m. by a visit to the production factory of Boots Pure Drug Co. Ltd.

The Scope of Automation in the Laboratory

Dr. G. Mattock stated that the application of automation in the analytical laboratory was primarily intended to reduce the man-hours required for analyses and so to make operations more efficient. In this sense it was not necessarily a requirement that the automatic technique be faster, but it must reduce the time demanded of an operator. In this sense it was justifiable to include newer instrumental methods of analysis, which themselves reduced the time required of the analyst, whether or not the instrument was fully automatic.

In the design of automatic instruments, it was neither adequate nor desirable merely to automate traditional methods: the technique used by the instrument must evolve from the requirement that a particular result was to be sought by automatic means, controlled by the acceptable accuracy (not the maximum possible accuracy) and ease of calibration. Further, it was important to emphasise that automation might be only partial to a complete analysis and was only valuable where it could eliminate one of the more time-consuming aspects.

Two different groups of automatic apparatus could therefore be identified—

(*i*) those performing a sequence of operations covering the entire analysis of one or several components, starting with the introduction of the untreated sample and ending with a record of the analysis, preferably on a repetitive basis, and

(*ii*) those performing only a part of the analysis and thus requiring manual intervention in intermediate stages.

Apparatus of type (*i*) was only economically useful when routine operations were carried out; type (*ii*) apparatus, while valuable for routine analysis, was also applicable to occasional analyses and was therefore probably of more general value.

Continuous analysers performing repetitive determinations on samples automatically fed into the apparatus were representative of class (*i*), and Dr. Mattock discussed them to illustrate how novel analytical principles could be introduced into automatic apparatus. Certain automatic titrators, while performing more orthodox operations, also illustrated class (*i*). There was a wide variety of apparatus in class (*ii*). Examples included automatic sampling devices and automatic titrators of set-end-point and curve-recording types. Also to be included were newer instrumental-measurement methods, which by their nature effected a reduction in the time necessary for a complete analysis, *e.g.*, techniques of X-ray fluorescence, photometry and direct electrometric measurement.

The Analysis of Commercial Sodium 2,2-Dichloropropionate (Dalapon Sodium Salt)

By D. C. GARRATT*

(*Standards Department, Boots Pure Drug Co. Ltd., Station Street, Nottingham*)

Several possible methods of determining the principal constituent of commercial sodium 2,2-dichloropropionate have been investigated and compared, and procedures for determining various impurities have also been established. The methods examined for determining 2,2-dichloropropionic acid include the mercury-precipitation method of Marquardt and Luce, a colorimetric method based on the formation of its 2,4-dinitrophenylhydrazone and a gas-chromatographic procedure.

A COMMERCIAL selective herbicide effective against couch grass (*Agropyron repens*) consists primarily of sodium 2,2-dichloropropionate together with relatively small amounts of associated chlorinated products and sodium pyruvate; the common name "dalapon" has been given to 2,2-dichloropropionic acid. Methods[1,2] for determining sodium 2,2-dichloropropionate are based on hydrolysis to pyruvic acid, but the reaction is not quantitative and impurities may also affect the results. Investigation into the reproducibility of any method with these variables is simple, but an assessment of accuracy depends on the purity of standards.

In order to study the accuracy of the chemical procedures and to investigate the quality of standards, it was considered that an alternative method was needed, and gas-chromatographic techniques based on the work of Crowshaw[3] were developed. With the gas-chromatographic methods a complete analysis of standards was possible, and, although the primary object was to assist in the assessment of the chemical techniques, good agreement was found between all methods for commercial products.

GAS CHROMATOGRAPHY—

The gas-chromatographic methods used were based on the separation of methyl esters of the chlorinated acids. Preliminary runs on esters prepared from commercial samples showed several peaks that were subsequently identified by comparison with samples of pure esters; in order of emergence from the column they were methyl monochloroacetate (MCA),

* This paper is published under the name of the head of the department, since many members of the staff contributed to the work recorded and discrimination for authorship would be unfair.

methyl 2-chloropropionate (MCP), methyl dichloroacetate (DCA), methyl 2,2-dichloropropionate (2,2-DCP), methyl trichloroacetate (TCA), methyl 2,3-dichloropropionate (2,3-DCP) and methyl 2,2,3-trichloropropionate (2,2,3-TCP). Methyl pyruvate is not resolved and is eluted at the same time as the methyl dichloroacetate.

To aid the identification of the components and to provide samples for use in standard mixtures pure esters were separated on a preparative scale under the following conditions—

Column length—6 feet.
Column diameter—1 inch.
Column temperature—100° C.
Stationary phase—20 per cent. of Apiezon M on ungraded Celite.
Carrier gas—Argon flowing at 600 ml per minute.
Flash-heater temperature—150° C.
Detector—Argon ionisation detector with 1700 volts applied.
Sample load—Approximately 0·5 g.

Determination of sodium 2,2-dichloropropionate

An internal-standard procedure was used and *o*-xylene was found to be suitable for the purpose, as it was eluted after 2,3-DCP and before 2,2,3-TCP, complete resolution being obtained. To prepare the methyl esters the extracted acids were treated with diazomethane in ether solution. The diazomethane was prepared by Boer and Backer's method,[4] which is given in the Appendix, p. 372.

Procedure—

Weigh accurately 1 g of sample into a 50-ml separating funnel, and dissolve in 3 ml of water. Add 10 ml of ether and then 5 ml of 10 per cent. v/v sulphuric acid, and shake the funnel vigorously. Separate the aqueous layer, and extract it with a further 5 ml of ether. Wash the combined ether extracts with 1 ml of water. Add diazomethane solution until the yellow colour persists, indicating an excess of reagent. Filter through paper, wash the paper with ether, and carefully evaporate the ether solution until a volume of about 10 ml is obtained. Cool, transfer to a 20-ml calibrated flask with small amounts of ethanol, add exactly 0·500 ml of *o*-xylene, and make up to volume with ethanol. Mix well.

Treat 0·85 g of standard sodium 2,2-dichloropropionate in exactly the same way for use as a standard, and chromatograph sample and standards under the following conditions—

Column length—5 feet.
Column diameter—4 to 5 mm.
Column temperature—94° C.
Stationary phase—20 per cent. of Apiezon M on 100- to 110-mesh Celite.
Carrier gas—Hydrogen - nitrogen mixture (4 + 1), 65 ml per minute.
Flash-heater temperature—150° C.
Detector—Thermal-conductivity detector.
Sample size—50 μl.
Recorder—1·0-mv full-scale deflection, chart speed 24 inches per hour.
Approximate inlet pressure—6 lb per sq. inch above atmospheric.
Outlet pressure—Atmospheric.

Under these conditions the column characteristics were—
Retention volume (*methyl 2,2-dichloropropionate*)—456 ml.
Retention volume (*o-xylene*)—952 ml.

From the curves obtained for both standard and sample, measure the areas under the methyl 2,2-dichloropropionate and *o*-xylene peaks. Calculate the 2,2-DCP content of the original sample from the equation—

$$\text{2,2-DCP, \%} = 85 \times \frac{AB_1}{SB}$$

where A = area under 2,2-DCP peak for sample,
B = area under *o*-xylene peak for sample,
S = area under 2,2-DCP peak for standard and
B_1 = area under *o*-xylene peak for standard.

Determination of impurities

The same sample solution was used as prepared for determining sodium dichloropropionate.

Standard mixtures of esters were prepared from pure samples, the relative amounts being chosen to cover the concentration range expected in the samples to be assayed. Suitable amounts of the esters were weighed into 20-ml calibrated flasks each containing 0·500 ml of *o*-xylene and then made up to volume with ethanol.

The chromatographic conditions were—

Column length—6 feet.
Column diameter—4 to 5 mm.
Column temperature—100° C.
Stationary phase—20 per cent. of Apiezon M on 100- to 110-mesh Celite.
Carrier gas—Argon, 35 ml per minute.
Flash-heater temperature—150° C.
Detector—Argon ionisation detector with 1700 volts applied.
Sample size—3 μl.

The retention volumes under these conditions were—

MCA	165 ml
MCP	209 ml
DCA	331 ml
2,2-DCP	407 ml
TCA	599 ml
2,3-DCP	736 ml
o-Xylene	880 ml
2,2,3-TCP	1530 ml

A standard curve for each minor component was prepared by using area ratios, with *o*-xylene as internal standard. During the run the recorder sensitivity was reduced by a factor of 10 during elution of the *o*-xylene. The peak area for each component in each standard mixture was measured, and the ratio to the peak area obtained for *o*-xylene was calculated. From these results standard curves were produced and were found to be reasonably reproducible from day to day.

It was convenient to use two separate instruments, one for determining dalapon and the other for determining impurities. Obviously this is not really necessary, since dalapon and its impurities can both be determined on the same sample by using a suitable standard mixture.

Chemical determination of sodium 2,2-dichloropropionate

Two basic methods are available. The first depends on hydrolysis to pyruvic acid and formation of a hydrazone, which may be either weighed or extracted and determined colorimetrically. The other depends on hydrolysis to pyruvic acid and simultaneous formation of a mercury complex, which is isolated and allowed to react with potassium iodide, and the liberated base is then titrated. In neither method is the hydrolysis complete; with the colorimetric method it is necessary to treat a standard simultaneously with the samples, but for the mercury method a factor may be employed.

Colorimetric hydrazone method—

The method is based on formation of the 2,4-dinitrophenylhydrazone after hydrolysis and measurement of its colour intensity after extraction into alkaline solution.[1,2]

The intensity and peak wavelength of the extracted hydrazone were found to vary according to the concentrations and proportions of the sodium carbonate and sodium hydroxide. This point was therefore examined in detail by carrying out determinations on a standard solution of freshly distilled pyruvic acid.

With sodium carbonate solution as the extraction medium it was found that peak absorption occurs at 370 mμ.

The $E^{1\%}_{1cm}$ values of the final solution were obtained from four separate determinations and found to be 2206, 2168, 2180 and 2188. Because of the variation, the effect of the amount of sodium carbonate present in the final solution was examined; the results were—

Volume of 20 per cent. w/v sodium carbonate per 200 ml, ml	5	10	15	20	25
$E^{1\%}_{1cm}$	2207	2207	2118	2066	2054

If sodium hydroxide is added to the sodium carbonate extract the peak absorption shifts to 445 mμ. Some workers have used the mixed alkalis to determine pyruvic acid, but opinion differs widely on the concentrations required. Work was carried out to determine the optimum normalities.

Extraction of the pyruvic acid 2,4-dinitrophenylhydrazone into sodium carbonate solution was carried out by the method described below in which two 5-ml portions of 20 per cent. w/v sodium carbonate solution are used, since this volume gave the highest $E^{1\%}_{1cm}$ values. Different volumes of N or 2 N sodium hydroxide were added to the carbonate extracts, and the solutions were diluted to 200 ml and read at the peak wavelength of 445 mμ after 10 minutes; the results are shown in Table I.

TABLE I

$E^{1\%}_{1cm}$ VALUES FOR SODIUM CARBONATE EXTRACTS

Concentration of measured solution with respect to sodium hydroxide, N	$E^{1\%}_{1cm}$	Wavelength of peak absorption, mμ
0·05	1510	440
0·075	1628	440
0·10	1666	440
0·13	1744	440
0·15	1782	440
0·18	1816	440
0·20	1862	445
0·23	1886	445
0·25	1912	445
0·30	1900	445
0·35	1906	445
0·40	1977	445
0·45	1990	445
0·50	2020	445
0·55	2040	445
0·60	2040	445
0·65	2040	445
0·75	2040	445
0·80	2040	445
0·90	2040	445
1·00	2020	445
1·20	1994	445

The stability of the colour developed with the final normality in sodium hydroxide of 0·75 was determined; the results were—

Time after adding sodium hydroxide, minutes	10	30	60	80	90	100	110	120
$E^{1\%}_{1cm}$	2040	2040	2040	2035	2027	2015	2002	1997

With the final normality in sodium hydroxide maintained at 0·75, the volume of 20 per cent. w/v sodium carbonate solution used was varied; the results were—

Volume of 20 per cent. w/v sodium carbonate solution, ml	5	6	8	10	11	12	13	15
Concentration of measured solution with respect to sodium carbonate, N	0·094	0·113	0·151	0·189	0·208	0·227	0·246	0·283
$E^{1\%}_{1cm}$	2014	2019	2027	2040	2040	2035	2030	2019

For maximum extinction values of reasonable stability, the normality of sodium hydroxide should fall between 0·55 and 0·90 and that of sodium carbonate between 0·19 and 0·23. It has been demonstrated that variations in laboratory temperature of from 0° to 37° C have no significant effect on the intensity of colour developed.

The procedure finally adopted for the colorimetric determination of sodium 2,2-dichloropropionate in commercial dalapon sodium salt is described below.

Preparation of 2,4-dinitrophenylhydrazine solution—Suspend 1 g of 2,4-dinitrophenylhydrazine in 10 ml of 2 N hydrochloric acid. Then add 5 ml of concentrated hydrochloric acid and finally, with agitation, a further 300 ml of 2 N hydrochloric acid. Filter the solution.

PROCEDURE—

Place 150 ml of distilled water and 2 ml of N sodium hydroxide in a 500-ml Erlenmeyer flask, and heat to boiling. Add to the boiling solution exactly 5 ml of a solution prepared by dissolving 1 g of sample in exactly 50 ml of distilled water, cover the flask with a watch-glass, and transfer immediately to an oven at 110° C. After 3 hours remove the flask, cool, and adjust the volume of the solution to 200 ml in a calibrated flask. Transfer 5 ml of this solution to a suitable separating funnel, add 2 ml of 2,4-dinitrophenylhydrazine solution, and mix thoroughly. Set aside for 20 minutes. Add 20 ml of toluene, and shake the funnel vigorously for 2 minutes. Remove the aqueous layer when clear, and discard. Remove excess of phenylhydrazine reagent by shaking the toluene with two 5-ml portions of 2 N hydrochloric acid, then remove traces of acid with 15 ml of water. Add exactly 10 ml of 10 per cent. w/v sodium carbonate solution, and shake the funnel vigorously for 2 minutes. Run the hydrazone into a 200-ml calibrated flask. Repeat the extraction with a further 10-ml portion of the sodium carbonate solution, followed by two washings with water, each of 15 ml, collecting all aqueous phases in the flask. Add 75 ml of 2 N sodium hydroxide, mix, dilute to volume with water, and mix again. Set aside for 10 minutes before measuring the extinction; read in either a 0·5- or 1-cm cell at 445 mμ within 1 hour of adding the hydroxide solution, and compare the colour with that obtained by treating a sample of pure sodium 2,2-dichloropropionate in the same way.

TITRIMETRIC MERCURY-COMPLEX METHOD—

The mercury method of Marquardt and Luce for sodium 2,2-dichloropropionate[5] was applied without modification. It should be noted, however, that the precipitated mercury complex may need to be washed with more water (75 to 100 ml) to free it from acid, and it is advisable to test the washings with litmus paper.

CHEMICAL DETERMINATIONS OF POSSIBLE IMPURITIES

SODIUM PYRUVATE—

As small amounts of sodium pyruvate are usually present in commercial samples of dalapon sodium salt, it is necessary to determine the amount, because results obtained by either the colorimetric or the titrimetric method for the principal constituent include this and must be corrected. The pyruvate is conveniently determined by the colorimetric procedure described above, a suitable weight of sample being used and the hydrolysis stage omitted.

SODIUM SALTS OF MONOCHLORO- AND TRICHLOROPROPIONIC ACIDS—

Marquardt and Luce described methods for the chemical determination of these two impurities, and it was of interest to compare the results with those obtained by gas chromatography.

The published procedure for the monochloropropionate was followed without alteration, but difficulty was experienced with the determination of the trichloropropionate. The directions given could not be followed clearly, since a number of inconsistencies are apparent in the published text.

The method used for trichloropropionate was similar to that given, but 0·5 g of sample and N potassium hydroxide were used. When carried out on laboratory-prepared mixtures with the standard sodium 2,2-dichloropropionate the results were—

Trichloropropionate added, %	5·5	5·4	2·2	2·2	0·0
Trichloropropionate found, %	5·7	5·5	3·4	3·0	1·6

It is concluded that results obtained for sodium 2,2,3-trichloropropionate by the chemical method are unsatisfactory, and if a determination of this component is required the gas-chromatographic method, which has given good recoveries on standard mixtures, should be used.

RESULTS

The methods described above were applied to commercial samples of dalapon sodium salt, and comparative results are recorded in Table II. Table III shows comparative results obtained in the routine application of these methods.

Some of the samples examined were known to contain a wetting agent and this did not interfere with the methods.

TABLE II

ANALYSIS OF SAMPLES OF DALAPON SODIUM SALT BY VARIOUS METHODS

Sample	Sodium 2,2-dichloropropionate *plus* sodium pyruvate, expressed as sodium 2,2-dichloropropionate, found by—		Sodium 2,2-dichloropropionate found by gas-chromatographic method, %	Sodium pyruvate found by colorimetric method, %	Sodium 2-monochloropropionate found by—		Sodium 2,2,3-trichloropropionate found by—	
	colorimetric method, %	mercury method, %			mercury method, %	gas-chromatographic method, %	mercury method, %	gas-chromatographic method, %
A	82·7, 83·3	83·6, 85·1, 84·2, 84·5, 84·1	82·9	0·64, 0·64	3·6	3·4	5·2, 4·3, 4·2	2·9
B	83·2, 81·6	83·3, 81·7, 83·5, 84·5, 83·7, 84·6, 83·8, 84·0	85·2	0·05, 0·05	7·5, 6·8	6·2	2·6, 2·5	1·1
C	83·2, 85·0	85·5, 85·1, 85·8, 86·1, 85·0	84·7	0·15, 0·15	4·2, 4·1	3·3	3·7, 4·3	2·0
D	87·2, 86·4	86·7, 86·6	87·1	0·13, 0·13	3·9, 3·2	3·8	3·2, 3·1	2·2
D *plus* 3 per cent. of added sodium pyruvate	88·4, 89·0	87·4, 88·1	86·9	3·31, 3·32	3·4, 2·7	4·0	2·6, 2·9	2·7

TABLE III

ANALYSIS OF SAMPLES OF DALAPON SODIUM SALT BY VARIOUS METHODS

Sample	Sodium 2,2-dichloropropionate *plus* sodium pyruvate, expressed as sodium 2,2-dichloropropionate, found by mercury method, %	Sodium 2,2-dichloropropionate found by gas-chromatographic method, %	Sodium pyruvate, as pyruvic acid, found by colorimetric method, %
E	85·2, 85·3, 84·0, 84·0, 85·9	84·3	0·15
F	84·1, 83·1, 84·9, 84·8	85·5	0·05
G	88·4, 88·4, 88·7	88·8	Negligible
H	84·4, 83·5	84·2	0·03
J	86·0, 86·1	88·4	0·04

CONCLUSION

A gas-chromatographic method for determining 2,2-dichloropropionic acid and the many possible impurities that may occur in commercial samples of dalapon sodium salt has been developed. Results for the main constituent have been compared with those obtained by chemical methods of assay, and, in general, agreement has been found to be satisfactory. The choice of method in any given instance would depend on the information required. If occasional samples are being analysed for 2,2-dichloropropionic acid content then the mercury-precipitation method of Marquardt and Luce is to be recommended. The colorimetric method requires less operator time and therefore offers advantages when many samples are to be examined simultaneously. When fuller information is required about the composition of the sample, the gas-chromatographic procedure is undoubtedly the method of choice.

Appendix

PREPARATION OF DIAZOMETHANE

REAGENTS—

Solution A—Dissolve 3·5 g of potassium hydroxide in 5 ml of water, and add 15 ml of absolute ethanol.

Solution B—Dissolve 15 g of *N*-methyl-*N*-nitroso-*p*-toluene sulphonamide in 85 ml of ether.

PROCEDURE—

Heat solution A in a 100-ml flask on a water bath maintained at 65° C. Connect to the flask a dropping funnel and a double-surface condenser leading into two 250-ml flasks in series, each containing 40 ml of ether and each cooled in an ice - salt mixture. Ensure that the inlet tubes to these flasks dip below the surface of the ether.

Add solution B slowly from the dropping funnel so that the rate of addition equals the rate of distillation. After all solution B has been added, add a further 10 ml of ether and continue the distillation until a colourless distillate is obtained.

Dry the ethereal solution of diazomethane contained in the first receiver for 4 hours with about 5 g of potassium hydroxide pellets, and then filter it through a Whatman No. 1 filter-paper into a suitable container. Fit a calcium chloride drying tube to the container, and store at 0° C. Under these conditions the solution is stable for about 1 week.

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Reactions of Steroids with Acetic Anhydride and Sulphuric Acid (the Liebermann - Burchard Test)

BY R. P. COOK

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The intensities of colours developed and the rates of reaction when various steroids (C_{18} to C_{30}) dissolved in acetic acid were treated with acetic anhydride and sulphuric acid (the Liebermann - Burchard test) have been investigated; cholesterol was used as standard. Only certain sterols (C_{27}, C_{28} and C_{29}) and some of their derivatives gave a typical blue-green colour.

The colour intensities produced, relative to that developed by cholesterol, could be grouped in five classes: (*i*) zero or slight, (*ii*) about half, (*iii*) equal to and either "fast" or "slow acting," (*iv*) about twice and either "fast" or "slow acting" and (*v*) four to six times greater, all being "fast acting."

The nature and conformation of the substituents in positions 3 and 7 of the sterol ring appear to be important.

THE Liebermann - Burchard test consists in adding to a sterol, dissolved in a suitable solvent, acetic anhydride containing a small amount of sulphuric acid, when a (usually) blue-green colour develops; a history of the test was given by Dam.[1] The test, used both qualitatively and quantitatively, is much employed in studies of sterols, particularly with cholesterol. This paper describes the comparative results obtained after testing a number of steroids and their derivatives dissolved in acetic acid, cholesterol (cholest-5-en-3β-ol) being used as an arbitrary standard. Some observations on cholesteryl esters dissolved in chloroform (many are insoluble in acetic acid) are also made.

The colours developed after 1·5 ("fast acting") and 35 minutes ("slow acting") were observed and measured (*cf.* paper by Moore and Baumann[2]).

Naming of compounds

The generic name "steroid" is used for compounds containing the perhydrocyclopentenophenanthrene nucleus with various substituents attached. In Table I (p. 375), the main headings are—

Sterols—These are steroids having a side-chain of eight, nine or ten carbon atoms, always with a hydroxyl group at position 3. In their systematic description, to avoid use of a large number of roots, cholestane has been taken as the parent compound; to this have been added (as prefixes, with the number in the steroid skeleton or in the side-chain) methyl, ethyl or hydroxyl substituents.

Stanols—These are fully saturated sterols.

Stenols—These are sterols having one double bond and one hydroxyl group in position 3.

Dihydroxystenols—These are sterols having one double bond and two hydroxyl groups.

Stenadi(tri)enols—These are sterols having two or three double bonds and one hydroxyl group.

Compounds defined by a page reference to Fieser and Fieser's "Steroids" are described in more detail in that book.[3] Systematic naming is generally in accordance with I.U.P.A.C. rules[4]; the trivial name or names follow the systematic name.

Method

Reagents and compounds—

All chemicals used were of analytical-reagent grade, and attention was paid to the removal of all but traces of water. The compounds tested were obtained from the sources listed in the Appendix, p. 380.

Cholesterol standard—Commercial cholesterol was recrystallised from glacial acetic acid by Fieser's method and then recrystallised from methanol; the purified compound was stored in dark bottles and kept at −15° C. Its melting-point was 149·5° to 150° C in a sealed capillary tube and 148° to 149° C in a Kofler block; its specific rotation, $[\alpha]_D^{20°}$, was −39·8° ($C = 1{\cdot}5$ in chloroform).

Procedure—

As only small amounts of certain compounds were available, samples of about 1 mg were accurately weighed in a tared 1-ml calibrated flask on a microbalance (sensitivity 0·01 mg). Glacial acetic acid was then added, with warming if necessary to ensure solution, and the contents of the flask were diluted to the mark. The cholesterol standard was similarly treated. The colour-forming reagent consisted of chilled acetic anhydride (19 volumes) to which was added 1 volume of concentrated sulphuric acid; the reagent was freshly prepared for each test. For the test itself, 1·0 ml of colour-forming reagent was added to 0·5 ml of steroid solution, and the solution was well mixed with a fine glass rod. The colour developed was observed visually after 1·5 and 35 minutes and after 20 hours. The intensity of colour was measured with an E.E.L. colorimeter (Evans Electroselenium Ltd.), the No. 205 red filter being used. Colour was developed at 20° ± 2° C in tubes shielded from direct light. Duplicate determinations were always made, and any discrepant values were re-determined. The results were calculated from the optical densities on a weight-to-weight relationship; the intensity of colour developed after 35 minutes by 0·5 mg of cholesterol was taken as 100. A numerical relationship was found, but, as the compounds had perforce to be tested in small batches over a period of 3 years, it was thought that a system of intensity rating indicating a broad comparative basis was more suitable than a whole-number relationship. With most of the compounds, the relationship is approximately equimolecular, notable exceptions being bi-steroids (see Table I, samples Nos. 54, 55 and 56) and cholesteryl esters.

Results

Sterols and related compounds—

The results for these substances are shown in Table I. For ease of presentation, references to similar studies on certain of the samples have been included.

TABLE I

REACTIONS OF STEROLS AND RELATED COMPOUNDS IN THE LIEBERMANN - BURCHARD TEST

The notation used for intensity of colour is based on the optical density compared with that developed by 0·5 mg of cholesterol (taken as 100): 0 = no reaction; S = 10 to 20; 0·5 C = 40 to 60; C = 80 to 140; 2 C = 160 to 240. Intensities greater than 400 are expressed to the nearest whole-number multiple of C. Colours and intensities after 20 hours are given only when stability or intensification was observed; in general, the colours faded to a green-blue of intensity about 0·5 C

Sample No.	Compound	Source No.*	Colour and relative intensity after— 1·5 minutes	35 minutes	Remarks	Reference†
Stanols—						
1	5α-Cholestan-3α-ol (epicholestanol)	9	0	Brown-green; S	3α axial	5
2	5α-Cholestan-3β-ol (cholestanol, dihydrocholesterol)	9	None	None	3β equatorial	
3	5β-Cholestan-3α-ol (epicoprostanol)	9	0	Pale blue; S	3α equatorial	
4	5β-Cholestan-3β-ol (coprostanol, coprosterol)	6, 13, 22	0	Green-blue; 0·5 C	3β axial	
5	4α-Methyl-5α-cholestan-3β-ol	14, 28	0	Green; S	Intensifies to about 0·5 C on standing	—
6	4,4-Dimethyl-5α-cholestan-3β-ol (dimethylcholestanol)	26	0	Green-blue; S		
7	5α-Cholestane-3β, 5α-diol	4	Blue-green; C	Deep blue-green; 2 C	3β equatorial, 5α axial	—
8	5α-Cholestane-3β, 7α-diol 3-acetate	1	Blue; C	Blue-green; 2 C	3β equatorial, 7α axial	6
9	5α-Cholestane-3β, 7β-diol 3-acetate	1	None	None	3β and 7β equatorial	
10	5α-Cholestane-3β, 5α, 6β-triol	11	0	Green; 2 C	3β equatorial, 5α and 6β axial	—
Stenols—						
11	Cholest-4-en-3β-ol (allocholesterol)	15	0	Blue-green; 2 C	3β equatorial	—
12	Cholest-5-en-3α-ol (epicholesterol)	15	0	Green; 2 C	3α axial	—
13	Cholest-5-en-3β-ol (cholesterol)	See text	0	Blue-green; C	3β equatorial	—
13A	Cholesterol hydrate	6	0	Blue-green; C	Intensity about 10 per cent. greater than for cholesterol	—
14	19-Norcholesta-5-en-3β-ol (norcholesterol)	29	0	Pink	—	7
15	5α-Cholest-6-en-3β-ol acetate	12	Blue-green; C	Blue-green; C	3β equatorial	—
16	5α-Cholest-7-en-3β-ol (lathosterol, Δ⁷-cholestenol)	9	Blue; 6 C	Blue; 6 C	3β equatorial	6
17	5β-Cholest-7-en-3β-ol (coprostenol)	2	Purple; C	Purple; C	3β axial	—

* See Appendix, p. 380. † See reference list, p. 380.

TABLE I (*contd.*)

Sample No.	Compound	Source No.*	Colour and relative intensity after— 1·5 minutes	35 minutes	Remarks	Reference†
Stenols (contd.)—						
18	4α-Methyl-5α-cholest-7-en-3β-ol (methostenol, lophenol)	15, 28	Deep blue; 5 to 6 C	Deep purple; 4 to 5 C	3β equatorial	8
19	4α-Methyl-5α-cholest-8-en-3β-ol	14	Blue-purple; 2 C	Blue-purple; 2 C	3β equatorial	9
20	4α-Methyl-5α-cholest-8(14)-en-3β-ol	14	Blue-purple; 2 C	Blue-purple; 2 C		
21	4α-Methyl-5α-cholest-14-en-3β-ol	14	Purple; 3 C	Purple; 3 C		
22	4,4-Dimethylcholest-5-en-3β-ol	4	Blue; 2 C	Green-blue; 3 C	3β equatorial	—
23	24a-Methylcholest-5-en-3β-ol (campesterol)	1	0	Blue-green; C		
24	24b-Ethylcholest-5-en-3β-ol (β-sitosterol)	1	Green; S	Green; C	3β equatorial	6
25	24-Ethylidenecholest-5-en-3β-ol (fucosterol)	1	0	Green; C	3β equatorial	—
Dihydroxystenols—						
26	Cholest-4-ene-3β, 6β-diol	21	Dark blue; 6 C	Green; 7 C	3β equatorial, 6β axial	—
27	Cholest-5-ene-3β, 4β-diol	12	Blue; 2 C	Green; 2 C	3β equatorial, 4β axial	—
28	Cholest-5-ene-3β, 7α-diol	12	Blue-green; 6 C	Blue-green; 6 C	3β equatorial, 7α axial	—
29	Cholest-5-ene-3β, 7β-diol	12	Blue; 6 C	Blue; 6 C	3β and 7β equatorial	—
30	Cholest-5-ene-3β, 24b-diol	9	0	Green; C	3β equatorial	—
31	Cholest-5-ene-3β, 25-diol	9, 24	0	Green; C		
32	Cholest-5-ene-3β, 26-diol	18	0	Blue-green; C		
Stenadi(tri)enols—						
33	Cholesta-5,7-dien-3β-ol (7-dehydrocholesterol)	20	Blue; 6 C	Green; 4 C	3β equatorial	—
34	Cholesta-5, 22-dien-3β-ol (22-dehydrocholesterol)	3	0	Blue-green; C		
35	Cholesta-5,24-dien-3β-ol (24-dehydrocholesterol, desmosterol)	3	0	Green; 0·5 C	3β equatorial	10
36	Cholesta-5,25-dien-3β-ol	3	0	Green; C	3β equatorial	—
37	Cholesta-8(9),22-dien-3β-ol (zymosterol)	1	Blue-green; C	Blue-green; C	3β equatorial	6 (for cholestenol analogues)
38	24b-Methylcholesta-8(14),22-dien-3β-ol ($\Delta^{8(14)}$-ergosterol)	4	Blue; 2 C	Green-blue; 2 C		
39	24b-Methylcholesta-5,22-dien-3β-ol (brassicasterol)	1	Green; S	Green; C	3β equatorial	—
40	24b-Methylcholesta-5,7,22-trien-3β-ol (ergosterol)	20	Blue; 6 C	Green; 4 C	3β equatorial	6
41	24b-Ethylcholesta-5,22-dien-3β-ol (stigmasterol)	1	0	Blue-green; C	3β equatorial	—
42	24b-Ethylcholesta-7,22-dien-3β-ol (α-spinasterol)	1	Blue; 5 C	Blue-green; 2 C	3β equatorial	6

* See Appendix, p. 380. † See reference list, p. 380.

TABLE I (*contd.*)

* See Appendix, p. 380. † See reference list, p. 380.

TABLE 1 (*contd.*)

Sample No.	Compound	Source No.*	Colour and relative intensity after— 1·5 minutes	35 minutes	Remarks	Reference†
Oxo compounds—						
43	5α-Cholestan-3-one (cholestanone)	9	None	None	—	—
44	5β-Cholestan-3-one (coprostanone)					
45	Cholest-4-en-3-one (Δ^4-cholestenone)					
46	Cholest-5-en-3-one (Δ^5-cholestenone)					
47	Cholest-7-en-3-one (Δ^7-cholestenone)	6	Purple; 4 C	Blue-green; 4 C	—	—
48	Cholesta-3,5-dien-7-one	4	0	Pale green; S	—	—
49	Cholesta-4,6-dien-3-one	4	0	Apple green; 0·5 C	Colour constant after 18 hours	—
50	24b-Methylcholesta-4,7,22-trien-3-one (ergosta-4,7,22-trien-3-one)	4	Fluorescent green; 2 C	Intense fluorescent green; 2 C	—	—
50A	Enol acetate of sample No. 50	4	Fluorescent green; 0·5 C	Intense fluorescent green; 2 C	—	—
51	Cholest-4-en-6β-hydroperoxy-3-one (Δ^4-cholestene-3-one-6β-hydroperoxide)	9	None	None	—	3 (p. 235)
Ketols—						
52	Cholest-5-en-3β-ol-7-one (7-oxo(keto)-cholesterol)	4	None	None	—	—
53	Cholest-5-en-3β, 26-diol-16,22-dione (kryptogenin)	4	None	Red-yellow fluorescence; S	—	—
Bi-steroids—						
54	Cholestenone pinacol	4	Purple; 2 C	Blue-green; 3 C	Multiply by 2 for molar comparison	11 (preparation and structure)
55	3,3-Bis(cholesta-3,5-dienyl)	4	Purple; C	Dark blue; 2 C	—	—
56	7-Oxocholesteryl acetate pinacol	4	Green; 0·5 C	Yellow; S	—	—
Miscellaneous—						
57	Cholest-5-en-3β-thiol (thiocholesterol)	19	0	Blue-green; 2 C	—	—
58	Cholesteryl methyl ether	6	0	Blue-green; C	—	—
59	Dicholesteryl ether	7	None	None	—	—
60	"Isocholesterol"	6	Yellow-green fluorescence	Yellow-green fluorescence	Mixture of trimethylsterols	3 (p. 364)
61	4,4,14-Trimethylcholest-8-en-3β-ol (Δ^8-lanosterol, dihydrolanosterol)	1	Yellow-green fluorescence	Yellow-green fluorescence	—	—

* See Appendix, p. 380. † See reference list, p. 380.

OTHER STEROIDS—

Representative steroids only were tested, but particular attention was paid to available compounds having a Δ^5, 3β-ol configuration (*i.e.*, 3β-hydroxyl-5-en). The sources of these samples are listed in the Appendix, p. 380.

Oestrogens—These have eighteen carbon atoms and are described on p. 463 of "Steroids."[3] Some samples of oestrone, oestradiol and oestriol from sources Nos. 16 and 25 tested under the standard conditions gave an immediate eosin-like fluorescent colour that later faded to yellow, but other samples gave negative results. With the latter, it was observed that a colour could be produced by adding excess of concentrated sulphuric acid.

Androgens—These have nineteen carbon atoms and are described on p. 519 of "Steroids."[3] 17β-Hydroxyandrost-4-en-3-one (testosterone) from source No. 17 gave no colour; 3β-hydroxyandrost-5-en-17-one (dehydroepiandrosterone) from source No. 15 gave a pale yellow; androst-5-ene-3β,17α-diol (source No. 15) gave an immediate deep red, changing after 30 minutes to yellow-brown; androst-5-ene-3β,17β-diol (source No. 15) gave no colour.

Gestagens—These have twenty-one carbon atoms and are described on p. 566 of "Steroids."[3] Pregn-4-ene-3,20-dione (progesterone; source No. 17) and its main reduction products gave pale yellow colours, as did the 3β,5-en compounds 3β-hydroxypregn-5-en-20-one and pregn-5-ene-3β,20β-diol (source No. 15).

Corticoids—These have twenty-one carbon atoms and are described on p. 603 of "Steroids."[3] No reaction was obtained with cortisol, cortisone and corticosterone from source No. 17. No samples of corticoids having 3β-hydroxyl-5-en structure were available for testing.

Cardenolides—These have twenty-three carbon atoms and are described on pp. 736, 752 and 754 of "Steroids."[3] Those tested were from sources Nos. 5 and 23 and had the 5β-configuration; the positions of the hydroxyl substituents are given after the trivial names, and the colours are those obtained after 30 minutes. Digitoxigenin (3β,14-diol), yellow; digoxigenin (3α,12α,14-triol), pale green; gitoxigenin (3β,14,16-triol), light yellow; strophanthidin (3β,5β,14-triol and CHO group at C_{19}), yellow, which intensified to about 0·5 C on standing.

Conessine—This has twenty-four carbon atoms and is described on p. 857 of "Steroids"[3]; it has the Δ^5 configuration, but a *N*-dimethyl group at position 3. A sample from source No. 4 gave a negative result.

Bile acids—These have twenty-four carbon atoms and are described on p. 422 of "Steroids."[3] The positions of the substituent hydroxyl groups in the parent cholanic acid are given after each trivial name and are followed by the source number; the colours are those formed after 30 minutes. Lithocholic (3α; No. 16), none; hyodeoxycholic (3α, 6α; No. 11), none; chenodeoxycholic (3α, 7α; No. 11), red-yellow, intensity about 10; deoxycholic (3α, 12α; No. 11), yellow-green, intensity about 10; hyocholic (3α, 6α, 7α; No. 8), none; acid II (α-muricholic; 3α, 6β, 7α; No. 8), none; acid I (β-muricholic; 3α, 6β, 7β; No. 8), none; acid IV (ω-muricholic; 3α, 6α, 7β; No. 8), none; cholic (3α, 7α, 12α; No. 16), red-yellow.

Sapogenins (*spirostans*)—These have twenty-seven carbon atoms and are described on p. 831 of "Steroids."[3] The main substituents, sources and colours after 30 minutes of the compounds tested were: tigogenin (5α, 3β-hydroxy; No. 10), pale yellow; hecogenin (5α, 3β-hydroxy, 12-oxo; No. 10), pale yellow; 9(11)-dehydrohecogenin (as for hecogenin, but with $\Delta^{9(11)}$; No. 4), pale yellow; diosgenin, (Δ^5, 3β-hydroxy; No. 10), pale yellow-green; kryptogenin is included in Table I (sample No. 53).

CHOLESTERYL ESTERS—

The cholesteryl acetate tested was from source No. 6; the others were from source No. 27 (preparation and properties described by Swell and Treadwell[12]). All the compounds were tested in chloroform; the esters of fatty acids up to C_{10} were also tested in acetic acid, those with longer chains being insoluble. These tests were carried out by Mr. A. El Sheltawy. Cholesterol in chloroform gives an intensity of 120, compared with 100 in acetic acid. The results are expressed as comparative to the standard in a particular solvent and are given as the cholesterol equivalent in the compound (calculated from the molecular weight).

Acetate and propionate, respectively, 60 and 90 (chloroform), 70 and 160 (acetic acid). Dimethylacetate, isobutyrate, n-valerate, isovalerate, DL-ethylmethylacetate, isohexanoate, octanoate and decanoate, about 130 (chloroform) and about 90 (acetic acid). Dodecanoate (laurate), tetradecanoate (myristate), hexadecanoate (palmitate), octadecanoate (stearate) and octadecenoate (oleate) were insoluble in acetic acid and each gave an intensity of about

130 in chloroform. A "fast" reaction, *i.e.*, development of colour after 1·5 minutes, was obtained with most of the esters in chloroform, and the intensity did not decrease greatly after 35 minutes. The benzoate behaved similarly.

Discussion of results

The production of chromogens, some of which are fluorescent, from steroids by relatively large amounts of the so-called Lewis acids, *i.e.*, electron acceptors (including with the conventional strong acids such compounds as antimony tri- and pentachlorides), is a commonly used qualitative and quantitative procedure.[13] The mechanism of the reaction or reactions is obscure,[14] and it was not our primary intention to study this, but to obtain information on the value and limitations of the Liebermann - Burchard test. The blue-green colour developed in this test by certain sterols and their derivatives is remarkable for the small amount of sulphuric acid needed, but only slight green-yellow colours are given by androgens, corticoids, bile acids, cardenolides and sapogenins. Oestrogens give, inconsistently, a red-green fluorescence, presumably owing to the phenolic nature of ring A. Trimethyl sterols (lanostane derivatives) give a yellow-green fluorescence.

Five broad divisions of colour development and intensity for sterols and their derivatives can be made. These divisions are set out below, the sample numbers referring to Table I.

(1) *Compounds producing a colour of slight or zero intensity*—This class includes 5α-cholestan-3β-ol (sample No. 2) and the corresponding mono- and dimethylstanols (samples Nos. 5 and 6), most oxosteroids (samples Nos. 43, 44, 45 and 46), 7-oxocholesterol (sample No. 52), 5α-cholestane-3β,7β-diol (sample No. 9) and dicholesteryl ether (sample No. 59). The methyl ether (sample No. 58), however, is reactive, possibly because of the relative ease of hydrolysis.

(2) *Compounds producing a colour intensity half that developed by cholesterol*—This class includes coprostanol (sample No. 4) and 24-dehydrocholesterol (sample No. 35).

(3) *Compounds producing a colour intensity about the same as that developed by cholesterol*—This class has two subdivisions, "fast-acting" and "slow-acting" compounds. The former subdivision includes stenols (samples Nos. 15 and 17) and zymosterol (sample No. 37). The latter subdivision includes all stenols having one double bond in position 5; the substitution of a hydroxy, methyl or ethyl group in the side-chain did not affect the intensity developed (compare results for samples Nos. 23, 24, 25, 30, 31 and 32), but the introduction of a double bond other than ethylidene into the side-chain appeared to cause lower intensities (compare samples Nos. 34, 35, 36 and 39, for which the intensities were 70, 50, 70 and 85, respectively). Cholesterol hydrate consistently gave an intensity 10 per cent. greater than that developed by cholesterol.

(4) *Compounds producing a colour intensity about twice that developed by cholesterol*—This class has the same subdivisions as Class 3. The "fast-acting" compounds include 4-mono- and 4,4-dimethylstenols having double bonds in positions 5, 8, 8(14) or 14 (samples Nos. 19, 20, 21 and 22) and the cholestanediols (samples Nos. 7 and 8). The "slow-acting" compounds include epicholesterol (sample No. 12) and thiocholesterol (sample No. 57).

(5) *Compounds producing a colour intensity five to six times that developed by cholesterol*—Members of this class are "fast acting," but the intensities developed decrease after 35 minutes. The compounds all have a double bond in position 7, either alone (samples Nos. 16, 18 and 42) or conjugated with a double bond in position 5 (samples Nos. 33 and 40); others are capable of conversion by dehydration to a conjugated diene (samples Nos. 26, 28 and 29). This "stenadiene" group will form a colour with acetic anhydride and zinc chloride as dehydrating agent,[15] a reaction not given by 7-en stenols; the intensity developed is similar to that developed by cholesterol, and the colour is stable. (This test is useful for differentiating between 7-en and 5,7-dien stenols.) Although oxosteroids are not generally reactive, Δ^7-cholestenone (sample No. 47) is "fast acting" (see also sample No. 50).

A few generalisations may be made, namely, that a blue-green colour is obtained only when the steroid has the complete nineteen carbon atoms of the steroid skeleton (for other examples, see paper by Pesez[7]) and a side-chain of at least eight carbon atoms. The nature

and conformation of the substituents at positions 3 and 7 are important. At position 3, the group may be a free or esterified hydroxyl group or a thiol group, and the axial conformation is apparently more reactive. Oxo substituents at position 3 are unreactive, except when there is a double bond at position 7. Cholesteryl esters are of interest in that they are apparently more reactive than the free sterol when in chloroform solution; this established fact[16] is still without explanation. At position 7, the presence of a double bond, or the possibility of dehydration to form one, confers increased reactivity; the presence of an oxo group decreases reactivity.

I thank the persons named in the Appendix for donating the steroids tested. Much of the experimental work was done by Miss D. Young, Miss Marian Greig, Mr. D. I. Cargill, Mr. A. D. Tait and Mr. A. El Sheltawy, to whom I am grateful. Dr. P. Bladon has kindly helped with timely and constructive advice. Part of the expenses was met by a grant from the Scottish Hospital Endowments Research Trust.

Appendix

The steroids tested were obtained from the sources listed below; unless otherwise stated, samples were kindly donated.

Sources

1. D. H. R. Barton, London.
2. C. A. Baumann, Madison, Wisconsin, U.S.A.
3. The late W. Bergmann, Yale University, New Haven, Connecticut, U.S.A.
4. P. Bladon, Royal College of Science and Technology, Glasgow.
5. Burroughs Wellcome & Co. Ltd., London, by courtesy of R. O. Thomson.
6. This laboratory; most of the compounds were recrystallised or prepared by A. D. Tait.
7. J. W. Cornforth, London.
8. E. A. Doisy, St. Louis University, St. Louis, Missouri, U.S.A.
9. L. F. Fieser, Harvard University, Cambridge, Massachusetts, U.S.A.
10. Glaxo Laboratories Ltd., London, by courtesy of T. F. Macrae.
11. G. A. D. Haslewood, London.
12. H. B. Henbest, Queen's University, Belfast, Northern Ireland.
13. R. N. Jones, Ottawa, Canada.
14. A. A. Kandutsch, Bar Harbor, Maine, U.S.A.
15. W. Klyne, London (Steroid Reference Collection).
16. L. Light & Co. Ltd., Colnbrook, Bucks., by purchase.
17. Merck & Co. Inc., Rahway, New Jersey, U.S.A., by courtesy of M. Tishler.
18. E. Mossetig, Bethesda, Maryland, U.S.A.
19. L. N. Owen, London.
20. Peboc Ltd., London, by purchase.
21. V. Prelog, Zurich, Switzerland.
22. The late O. Rosenheim, London.
23. Sandoz, Basle, Switzerland, by courtesy of R. A. Ellis.
24. Schering Corporation, Bloomfield, New Jersey, U.S.A., by courtesy of A. Ryer.
25. G. D. Searle & Co., Chicago, Illinois, U.S.A., by courtesy of V. A. Drill.
26. R. Stevenson, Brandeis University, Waltham, Massachusetts, U.S.A.
27. C. R. Treadwell, Washington, D.C., U.S.A.
28. W. W. Wells, Pittsburgh, Pennsylvania, U.S.A.
29. U.C.L.A.F., Paris, by courtesy of P. Poirier.

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The Iodimetric Determination of Acetaldehyde Bisulphite

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Methods for the iodimetric titration of acetaldehyde bisulphite in the presence of borate buffer solution give low results. This is shown to be caused by oxidation of sulphite by dissolved oxygen and can be overcome by a simple modification to the usual procedure.

The determination of acetaldehyde is of considerable importance in the study of wine and cider, and the methods generally used are based on iodimetric titration of acetaldehyde bisulphite in slightly alkaline solution. This procedure has been attributed[1] to Tomoda,[2] although the same principle was previously used by Clausen[3] in 1922 and was adopted by Friedmann, Cotonio and Schaffer[4] for determining lactate in blood after oxidation to acetaldehyde.

Tomoda allowed the acetaldehyde to react in aqueous solution with a slight excess of sodium bisulphite and, after 15 minutes, destroyed the excess of bisulphite by adding iodine, with starch as indicator, until a pale-blue end-point was reached. The acetaldehyde - bisulphite compound was then caused to dissociate by saturating the solution with sodium hydrogen carbonate. This increased the pH to about 8, and, although the complex was only dissociated to the extent of a few per cent., the rate of dissociation was such that the liberated bisulphite could be titrated with iodine.

Jaulmes and Espezel[1] modified this method in order to determine smaller amounts of acetaldehyde in distillates from wine. They showed that the rate of combination of acetaldehyde with sodium bisulphite was dependent on pH and was maximal at pH 7; they therefore carried out the addition reaction at this pH. Their other modification was to replace sodium hydrogen carbonate by an alkaline borate solution, which they said produced a sharper end-point in the titration with iodine.

Jaulmes and Espezel's method has become widely accepted[5] for determining acetaldehyde in wines, both in its original form[6,7] and with minor modifications.[8,9,10] Joslyn and Comar[7] found that recovery of acetaldehyde was only about 90 per cent., both from pure solution and after distillation from wine. Ribereau-Gayon and Peynaud[8] claimed that the error by their method was less than 1 per cent., but did not give results for the recovery of added acetaldehyde. They emphasised that the final titration with iodine should be carried out immediately after the alkaline borate solution had been added, otherwise sulphur dioxide was lost by oxidation. Paul[9] mentioned difficulty with the iodimetric end-point when sodium hydrogen carbonate was used and devised a sodium carbonate - sodium tetraborate buffer; he claimed that his results for standard solutions of acetaldehyde agreed well with the amounts known to be present, but did not report actual figures. Dukhovnyi[10] used a method similar to that described by Ribereau-Gayon and Peynaud and reported an error of less than 3 per cent. in the recovery of acetaldehyde added to wine; however, the presence of ethanol in the distillates may have protected the sulphur dioxide from oxidation.

Our investigation was initiated because Ribereau-Gayon and Peynaud's method was found to give low recoveries (about 90 per cent.) of acetaldehyde from standard solutions. This and other methods were therefore examined in an attempt to find the source of this error and a means to remove it.

Experimental

The four methods examined are essentially the same in principle, and the various stages are outlined below.

(*i*) The acetaldehyde solution is allowed to react for 20 minutes with a large excess of sodium bisulphite solution in phosphate buffer (pH 7) at room temperature.

(*ii*) The solution is acidified (pH 1 to 2), and the excess of sodium bisulphite is removed by adding 0·1 N iodine to a pale-blue end-point (starch as indicator). The rate of dissociation of the acetaldehyde - bisulphite compound is extremely slow at this pH.

(*iii*) The pH is then increased to about 8 by adding alkaline borate solution (or solid sodium hydrogen carbonate in Tomoda's method[2]). At this pH, the rate of dissociation of the acetaldehyde - bisulphite compound is greatly increased.

(*iv*) The liberated sulphite is immediately titrated with a dilute standard solution of iodine.

The methods differed mainly in the volume and composition of the alkaline borate solution used in stage (*iii*), and this affects the pH at which the final titration is carried out. The volumes of the various solutions used in this investigation are shown in Table I.

Table I

Amounts of reagents used

The volume of sample solution used throughout was 25 ml

Reagent	Volume of reagent used in— method A,*	method B,†	method C,‡	method D,§
Stage (i)—	ml	ml	ml	ml
Buffer solution, pH 7	50	25	25	25
Sodium bisulphite solution, 2 per cent. w/v ..	10	5	5	5
Stage (ii)—				
Hydrochloric acid, 25 per cent. v/v ..	10	5	2·5	5
Stage (iii)—				
Alkaline borate solution	100	*x*	62·5	$NaHCO_3$ (solid)

* Jaulmes and Espezel's method.[1] Water (75 ml) was also added at stage (*i*).

† Ribereau-Gayon and Peynaud's method.[8] In stage (*iii*), the solution was titrated with the alkaline borate solution to a phenolphthalein end-point; the value of *x* was approximately 20 ml.

‡ Paul's method.[9] For comparison with the other methods, the volumes used were 2·5 times greater than those originally recommended.

§ Tomoda's method.[2] In the original procedure, buffer solution was not used in stage (*i*); it was added here to ensure completeness of reaction and for the sake of uniformity.

Reagents—

With the exception of sodium metabisulphite, all materials used were of analytical-reagent grade.

Phosphate buffer solution, pH 7—Prepare a solution containing 15 g of disodium hydrogen orthophosphate, $Na_2HPO_4.12H_2O$, and 3·35 g of anhydrous potassium dihydrogen orthophosphate per litre.

Sodium bisulphite solution, 2 per cent. w/v—Freshly prepare this reagent from sodium metabisulphite having a purity of 93 per cent.

Hydrochloric acid solution, 25 per cent. v/v.

Alkaline borate solution for use in method A—Dissolve 16 g of sodium hydroxide and 8·75 g of boric acid in water, and dilute to 1 litre.

Alkaline borate solution for use in method B—Dissolve 40 g of sodium hydroxide and 30 g of boric acid in water, and dilute to 1 litre.

Alkaline borate solution for use in method C—Dissolve 114 g of sodium carbonate decahydrate and 8·75 g of boric acid in water, and dilute to 1 litre.

Sodium hydrogen carbonate.

Iodine solutions, 0·1, 0·02 *and* 0·01 N.

Starch indicator—Freshly prepare a 0·5 per cent. w/v solution of soluble starch.

Standard acetaldehyde solution—Prepare acetaldehyde from paraldehyde by slow distillation in the presence of 1 per cent. v/v of concentrated sulphuric acid. Collect the distillate in an ice-cooled flask, store it in a refrigerator, and re-distil small portions immediately before use.

Prepare standard solutions by dissolving 1·5 g of sodium metabisulphite in 20 ml of water in a 25-ml calibrated flask, accurately weigh the flask and its contents, add 0·8 ml of re-distilled acetaldehyde, by pipette, and again weigh.

Dilute the solution to the mark, and use suitable dilutions of this solution for analysis. (The incorporation of sodium bisulphite in slight excess over the amount required to react with the acetaldehyde was designed to prevent loss of acetaldehyde by volatilisation.)

Results and discussion of the method

A solution containing 0·4814 g of acetaldehyde per litre was analysed by the four methods. Recoveries of acetaldehyde from 25-ml aliquots, each containing 12·04 mg of acetaldehyde, are shown in Table II, together with the pH values at different stages.

Table II

Recoveries of acetaldehyde by the four methods

Each sample (25 ml) contained 12·04 mg of acetaldehyde

Method	pH at stage (*i*)	pH after acidification	pH after titration	Acetaldehyde found, mg	Recovery, %
A	6·68	1·34	8·30	11·35, 11·23, 11·16 (mean 11·25)	93·4
B	6·64	1·06	8·48	10·90, 11·20, 11·05 (mean 11·05)	91·7
C	6·64	1·46	9·30	9·88, 9·55, 9·86 (mean 9·76)	81·1
D	6·64	1·06	7·48	12·08, 12·12, 12·07 (mean 12·09)	100·4

Recovery was inversely related to the pH of the solution after titration with iodine. The low recoveries when methods A, B and C were used were not due to incomplete dissociation of the acetaldehyde - bisulphite compound, as the rate at which the iodine was decolorised during these titrations was almost instantaneous and much faster than in method D. The losses were attributed to oxidation of sulphite, presumably by oxygen already dissolved in the solution, since all titrations were carried out immediately after the alkaline borate solution had been added.

Inhibition of sulphite oxidation by isopropyl alcohol—

Oxidation of sulphite by dissolved oxygen is a chain reaction and can be inhibited by the use of isopropyl alcohol.[11] The protective action of isopropyl alcohol on the iodimetric titration of sulphite at pH 8·5 was studied as follows. Each of a series of 10-ml portions of 0·01 N sodium bisulphite *plus* 5 ml of 25 per cent. v/v hydrochloric acid and a known volume of isopropyl alcohol was titrated with the alkaline borate solution used in method B to a phenolphthalein end-point, and the sulphite was then immediately titrated with 0·01 N iodine. The results were—

Isopropyl alcohol present before titration with iodine solution, % v/v	0·0	0·3	1·6	3·1	13·7
Recovery of sulphite, %	56	92	96	96	98

from which it can be seen that even 0·3 per cent. of isopropyl alcohol markedly inhibits the oxidation of sulphite at this pH.

A solution containing 0·4920 g of acetaldehyde per litre was then analysed in the presence of sufficient isopropyl alcohol (added before the alkaline borate solution) to give a concentration of 10 per cent. v/v in the solution to be titrated with iodine. The recoveries of acetaldehyde by methods B and C were 97·7 and 98·9 per cent., respectively, thereby confirming that the losses shown in Table II were caused by oxidation of sulphite by dissolved oxygen.

Effect of pH on rate of oxidation of sodium bisulphite solution—

The results in Table II suggest that the oxidation of sulphite is critically influenced by the pH of the solution. This reaction has been studied in detail by Abel,[12] who found

that the rate of oxidation was proportional to the concentration of sulphite and inversely proportional to the square root of the concentration of hydrogen ions. The influence of these two factors under conditions similar to those of the determination of acetaldehyde was examined as described below.

Approximately 45 ml of a buffer solution (containing 25 ml of the pH 7 buffer, 5 ml of the 25 per cent. hydrochloric acid and sufficient alkaline borate solution to give the required pH) were added to 20 ml of 0·01 N sodium bisulphite. After it had been set aside for a measured time, the mixture was poured into 25 ml of 0·01 N iodine acidified with 5 ml of N hydrochloric acid, and the residual iodine was titrated with 0·01 N sodium thiosulphate. In the control titration, representing contact with the buffer solution for zero time, 45 ml of water were used instead of the buffer. The results of this experiment are shown in Table III.

TABLE III

EFFECT OF pH ON OXIDATION OF SULPHITE

Time of contact with buffer solution	Loss of sulphite with buffer solution of—			
	pH 6, %	pH 7, %	pH 8, %	pH 9, %
10 seconds	8·7	22·4	29·0	37·0
30 seconds	13·7	33·8	37·4	40·2
1 minute	21·6	37·5	39·8	44·0
2 minutes	31·4	39·3	41·8	43·4
5 minutes	34·2	41·0	46·0	48·6

At pH 7 and above, most of the oxidation occurred within 30 seconds and was presumably caused by oxygen already in solution. The slower oxidation occurring when the solution was set aside may be attributed in part to the further absorption of atmospheric oxygen and also to the progressively decreasing concentration of sulphite ions. The effect of concentration of sulphite was demonstrated by repeating the experiment with 5 ml of 0·01 N sodium bisulphite instead of 20 ml. At pH 7, only 15 per cent. of the sulphite was oxidised in 10 seconds and 26 per cent. in 5 minutes.

The effect of concentration of sulphite on the rate of oxidation would also account for the fact that the oxidative loss of sulphite in the form of sodium bisulphite (see Table III) is much greater than that observed in the determination of acetaldehyde (see Table II), in which only a small fraction, dependent on the pH, of the total sulphite is ionised. An experiment similar to that giving the results in Table III was carried out with 20-ml aliquots of 0·01 N acetaldehyde - sodium bisulphite; after contact for 1 minute with buffers of pH 7, 8 and 9, the losses of sulphite were 4·8, 7·0 and 7·8 per cent., respectively.

MODIFICATION TO PROCEDURE—

The factors responsible for low results in the iodimetric titration of the acetaldehyde - bisulphite compound can be summarised as follows.

(*i*) When the pH is increased by adding alkaline borate solution, the sulphite liberated becomes susceptible to oxidation by dissolved oxygen.

(*ii*) This is a chain reaction, the rate of which (*a*) increases with increasing pH, (*b*) is proportional to the concentration of sulphite ions and (*c*) is inhibited by the presence of isopropyl alcohol.

Oxidative loss of sulphite can be largely overcome by adding isopropyl alcohol before the alkaline borate solution, but some slight loss may still occur, depending on how long the solution is set aside before titration with iodine. However, this oxidative loss can be completely prevented by adding 90 to 95 per cent. of the iodine required in the titration before the alkaline borate solution is added. In this way, the sulphite, as it is progressively liberated by increasing the pH, reacts immediately with the iodine and is not subject to oxidation by dissolved oxygen. A preliminary determination by the normal procedure is necessary to find the approximate amount of iodine needed in the titration. Huff[13] used a similar procedure to avoid loss of sulphite in the determination of hydroxyacetone. It is not clear, however, whether or not the loss of sulphite was caused by oxidation, since he used sodium hydrogen carbonate to liberate the bound sulphite, and, further, this modification was apparently not necessary with other carbonyl compounds.

The same standard acetaldehyde solution used for the experiments reported in Table II was analysed by methods A, B and C, with this modification. Recoveries were 100·2, 100·2 and 100·7 per cent., respectively, with close agreement between triplicate titrations.

It is noteworthy that method D, in which sodium hydrogen carbonate is used, also gives complete recovery of acetaldehyde, although the final pH of 7·5 is such that some oxidative loss of sulphite would be expected. That this does not occur is probably due in part to the fact that titration with iodine is usually begun before all the sodium hydrogen carbonate has dissolved, so that, effectively, the sulphite reacts with the iodine as soon as it is liberated. In this way, method D approximates to the modified procedures involving use of alkaline borate solution. Another factor minimising oxidative loss of sulphite is that the dissolved oxygen may be largely removed from solution by the vigorous evolution of carbon dioxide. It is unfortunate that this method has the disadvanatages of slow liberation of sulphite and a less distinct end-point, as it is otherwise reliable and accurate.

Since all three methods in which alkaline borate solution is used give complete recovery of acetaldehyde the choice becomes a matter of convenience. Method B can be simplified by using a fixed volume (about 23 ml) of alkaline borate solution without recourse to titration with phenolphthalein as indicator. This slight excess of alkaline borate solution does not affect the modified procedure.

The possibility was considered that some of the iodine might react with the acetaldehyde liberated concomitantly with the sulphite. Solutions of acetaldehyde (2·5 mg) in 20 ml of buffer solution (pH 8) were set aside in contact with 10 ml of 0·01 N iodine, acidified and then titrated with sodium thiosulphate solution. After contact for 2 minutes, the loss of iodine was barely detectable and after 15 minutes it was only 0·5 ml of 0·01 N iodine. The oxidation of acetaldehyde by iodine is therefore negligible under the conditions of these determinations.

Finally, a standard solution of acetaldehyde was analysed, ten replicate determinations at each of two concentrations (11·8 and 2·36 mg per 25 ml) being made by the modified procedure of method B. The mean recoveries were 100·0 and 100·3 per cent. and coefficients of variation were 0·112 and 0·211 per cent., respectively.

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Determination of Cadmium in Rocks by Neutron-activation Analysis

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A neutron-activation method has been developed for determining 0·1 to 1 p.p.m. of cadmium in rocks. It depends on the separation of ^{115}Cd (half-life 55 hours) with added cadmium carrier from a solution of the irradiated powdered rock by successive precipitations with trimethylphenylammonium iodide, 5,6-benzoquinoline and brucine. The precipitates are re-dissolved in a mixture of sulphuric and perchloric acids, and the cadmium is finally mounted for beta-counting as its brucinium salt. Standards are prepared by irradiating microgram amounts of cadmium acetate. The method has been applied to a suite of rocks from the Skaergaard Intrusion, Greenland, and also to the international standard rock samples G-1 and W-1.

Because of the lack of sufficiently sensitive analytical methods, little is known of the occurrence of cadmium in igneous rocks other than those rich in sulphide minerals. A colorimetric method[1] has yielded most of the results hitherto available, but it is not claimed to be highly accurate. Cadmium has also been determined by spectrography[2,3,4] and polarography[5,6,7]; the excitation of cadmium in flames has recently been reviewed.[8]

Neutron-activation analysis has been applied to the determination of cadmium in vinylite resins,[9] which were found to contain 100 to 400 p.p.m. of the element, and DeVoe and Meinke[10] have studied the radiochemical separation of cadmium from about twenty elements. The results obtained when the method described here was applied to rocks and minerals of the Skaergaard Intrusion have already been discussed[11] from a geochemical standpoint.

Nuclear chemistry

From a consideration of the isotopic composition of natural cadmium and the half-lives of the expected (n,γ) products, it can be concluded that the only active nuclides present in significant amount in a sample of cadmium after irradiation for 1 week in a flux of thermal neutrons and then decay for 24 hours should be ^{115}Cd (half-life 55 hours) and its metastable form ^{115m}Cd (half-life 43 days). Since the cross-section for formation is eight times greater for ^{115}Cd than for ^{115m}Cd and since the shorter-lived nuclide attains 87 per cent. of its saturation yield in 1 week (against 9 per cent. for ^{115m}Cd), it follows that the observed activity of a sample of cadmium treated as indicated above should be mainly due to ^{115}Cd. This conclusion is substantiated by the behaviour of the cadmium activity separated from rock samples and standards, and, since a half-life of 2·3 days allows ample time for chemical treatment, the determination of traces of cadmium by neutron activation seems feasible.

The decay scheme of ^{115}Cd involves a radioactive daughter isotope, ^{115}In (half-life 4·5 hours), so that the total activity of a freshly prepared sample of ^{115}Cd increases during the first few hours. In general, for the scheme—

$$\text{A (active)} \xrightarrow{\lambda_A} \text{B (active)} \xrightarrow{\lambda_B} \text{C(stable)}$$

where λ_A and λ_B are the respective disintegration constants, if λ_A is less than λ_B maximum total activity occurs at a time after separation of pure A given by the equation[12]—

$$t_{max.} = \frac{1}{\lambda_B - \lambda_A} \ln\left(\frac{\lambda^2_B}{2\lambda_A\lambda_B - \lambda^2_A}\right)$$

In this instance, $t_{max.}$ is 12·9 hours. After maximum activity has been reached, transient equilibrium is established between parent and daughter isotopes, and the total activity decays smoothly with the parent's half-life. In a method based on ^{115}Cd, therefore, it is necessary to wait about 24 hours after separation before the sources are counted.

Consideration must be given to the possible occurrence of nuclear reactions yielding ^{115}Cd in samples of rock, but not in standards, thereby invalidating comparison between the two; three such reactions are—

(*i*) $^{115}_{49}In\ (n,p)\ ^{115}Cd$

(*ii*) $^{118}_{50}Sn\ (n,\alpha)\ ^{115}Cd$

(*iii*) $^{235}U\ (n,\text{fission})\ ^{115}Cd$

The types of reaction involved in (*i*) and (*ii*) rarely occur by the action of thermal neutrons on elements of atomic number greater than about 40,[13] and reaction (*iii*) is unlikely to be significant, since cadmium lies in the trough of the uranium fission yield curve. Experiments with indium, tin and uranium monitors showed that the maximum error that could be caused by the presence in a sample of rock of all three of these elements in concentrations similar to that of cadmium was about 0·5 per cent.; this was negligible for our purpose.

Other potential sources of error are self-shielding and self-absorption. Self-shielding during irradiation is likely to be exceptionally severe for cadmium, owing to its high capture cross-section for thermal neutrons, and so the amount of a pure cadmium compound that can be used as an irradiation standard is expected to be correspondingly low. If self-shielding occurs, a large sample exhibits a lower specific activity than does a small one. Two separate irradiations of cadmium acetate dissolved in de-mineralised water, showed that only little self-shielding occurred in the range 0·01 to 1·0 μg of cadmium; the results were—

Irradiation No.	1				2			
Cadmium irradiated, μg	0·926	0·672	0·107	0·0115	0·908	0·474	0·095	0·0093
Activity per μg, counts per minute	3594	3466	3883	3899	3835	3973	4007	4103

If self-shielding occurs in the samples of rock, the lower specific activity of a large sample of a particular rock will lead to an under-estimate of its cadmium content when compared with the result from a smaller sample irradiated at the same time. The results in Table I do not show this effect.

TABLE I

REPLICATE RESULTS FOR SAMPLES OF ROCKS

Sample No.	Type of rock	Weight of sample, mg	Cadmium found, p.p.m.
E.G. 5321	Gabbro	92·6	0·32
		208·6	0·34
E.G. 4507	Gabbro	105·5	0·125
		216·1	0·131
W-1	Diabase	101·3	0·34
		116·2	0·30
		227·3	0·33
		144·5*	0·34

* Separate irradiation.

Self-absorption is severest when low-energy beta-particles are counted. The results below show that the apparent activity of a source of cadmium "brucinate" having constant surface area slowly decreases as its thickness (proportional to the amount of cadmium present) increases. To minimise this cause of error, it was desirable to restrict the thickness of these sources to the equivalent of 5 mg of cadmium or less.

Cadmium present, mg	15	12	9	6	3
Activity, counts per minute	235	242	247	273	267

ANALYTICAL CHEMISTRY

In developing a method for isolating radiochemically pure cadmium from the complex mixture of isotopes produced in a rock by irradiation with neutrons, a survey was made of the organic precipitants available. The most popular gravimetric reagents for cadmium have been[14] anthranilic acid, quinaldinic acid and oxine, but these were insufficiently specific for our purpose. 2-(*o*-Hydroxyphenyl)benzoxazole is said to be highly specific for cadmium,[15] but this has been questioned[10,16]; further, the necessary careful adjustment of pH is tedious.

In the presence of potassium halide, brucine sulphate forms a white precipitate with cadmium[17]; this brucinium salt contains only 9·2 per cent. of cadmium and so is a convenient means of handling a small amount of the element. Cobalt, nickel, copper, iron, chromium and zinc form similar precipitates. 5,6-Benzoquinoline and trimethylphenylammonium iodide behave alike as precipitants for cadmium[18] and are not subject to interference from nearly all the elements just mentioned. There is interference from copper, lead, zinc and bismuth, however, but these elements can be displaced by the addition of elemental iron. Hexamine allylo-iodide has been recommended for use with cadmium,[19] but the results are not always satisfactory.[20]

On the basis of this survey and experiments to ascertain the characteristics of the precipitates involved, 5,6-benzoquinoline, trimethylphenylammonium iodide and brucine were selected as precipitants, with the brucinium salt as the "plating-out" form. Aqua regia was found to be unsatisfactory for re-dissolving the precipitates, but a mixture of sulphuric and perchloric acids (17 + 3) gave good results. It was ascertained that the precipitated cadmium "brucinate" was of reproducible known composition.

To assist in attaining radiochemical purity, scavenge steps with ferric hydroxide and silver iodide were included. Together with the iron solution were added manganese and magnesium carriers, which, when made alkaline, remove ^{56}Mn and ^{114m}In, respectively[21]; otherwise, indium closely follows cadmium. Holdback carriers were used to assist the purification each time a cadmium compound was precipitated.

PREPARATION OF STANDARDS AND SAMPLES FOR IRRADIATION—

AnalaR cadmium acetate was chosen for preparing the irradiation standards in preference to the chloride, which gives rise to excessive extraneous activity, or the metal, which is not available in a pure condition and would suffer from self-shielding. About 0·2 ml of cadmium acetate solution, containing 0·01 to 1·0 μg of cadmium, is introduced into a tared silica ampoule. After the ampoule has been re-weighed and its contents evaporated to dryness, it is sealed and sent for irradiation. As the concentration of the acetate solution is known, the exact amount of cadmium taken can be calculated. After return from irradiation, each ampoule is rinsed in a warm dilute solution of cadmium, dried and cut open. The portions are immersed in hot water for about 30 minutes, with thorough rinsing, and the solution is made up with washings to 50 ml.

Samples of rock are ground to a fine powder in an agate mortar and sealed in lengths of silica or (more conveniently) polythene tubing marked with black Chinagraph pencil; 100 to 300 mg of powder is a convenient amount. The tubes should be only two-thirds full, so that, after irradiation, the ends can be cut off and the powder removed by tapping without loss.

Samples and standards were irradiated for 1 week in a flux of 10^{12} neutrons per sq. cm per second.

METHOD

REAGENTS—

Trimethylphenylammonium iodide solution—Prepare an aqueous 3 per cent. solution of the reagent, and filter to remove dark insoluble matter.

Trimethylphenylammonium iodide wash solution—Dissolve 1 g each of the reagent and potassium iodide in 200 ml of water, and filter.

Brucine sulphate solution—Dissolve 1 g of brucine in 100 ml of cold 20 per cent. sulphuric acid; alternatively, use an aqueous 1 per cent. solution of brucine sulphate. The yellow colour that develops when the solution is stored is not detrimental.

Brucine wash solution—Mix 40 ml of the brucine sulphate solution, 15 ml of 20 per cent. potassium bromide solution and 100 ml of water.

5,6-*Benzoquinoline solution*—Dissolve 2·5 g of the reagent in 100 ml of 0·2 N sulphuric acid.

5,6-*Benzoquinoline wash solution*—Mix 10 ml of the 5,6-benzoquinoline solution, 10 ml of 0·2 N potassium iodide and 150 ml of water, and add a few crystals of sodium sulphite.

Holdback carrier solution A—Prepare to contain approximately 1 mg each of lead, copper, bismuth, mercury, arsenic and tin per ml, as nitrates or chlorides.

Holdback carrier solution B—Prepare to contain approximately 1 mg each of cobalt, nickel, manganese, chromium, magnesium, zinc and sodium per ml, as nitrates or chlorides.

Holdback carrier solution C—Prepare to contain 1 mg each of copper, magnesium, manganese, potassium and sodium per ml, as chlorides or sulphates, but not as nitrates.

Perchloric - sulphuric acid mixture—Add 3 ml of 60 per cent. perchloric acid to 17 ml of concentrated sulphuric acid, with stirring.

Iron-scavenge solution—Prepare to contain 10 mg of ferric iron (as ammonium ferric sulphate), 5 mg of manganese (as potassium permanganate) and 5 mg of magnesium (as sulphate) per ml.

PROCEDURE FOR SAMPLES OF ROCK—

Take normal precautions for handling moderate levels of radioactivity, and begin work as soon as the irradiation can is returned. Bring the powdered rock into solution in the presence of 15 mg of cadmium, usually by Rafter's method[22] (dissolve the cake resulting from the fusion with sodium peroxide in the minimum volume of water containing the cadmium carrier as sulphate). To the solution add 5 ml of concentrated hydrochloric acid, and evaporate to incipient dryness; repeat this step, adding 1 ml of concentrated nitric acid if necessary, until only silica has not been dissolved. To the moist solid add 2 ml of holdback carrier solution A, 1 g of sodium sulphite, 10 ml of 20 per cent. sulphuric acid (7 N) and a few bright iron nails. Cover, and boil for about 20 minutes until the yellow or green of the solution is obscured by the dark colour of iron and the displaced metals. Decant the hot mixture into a centrifuge tube, spin, and decant the supernatant solution (or remove it by filtration). Wash the solids by centrifugation with hot water, and combine the solutions until 25 to 35 ml of mixture approximately 2 N in sulphuric acid are obtained. Add 1 g each of potassium iodide and sodium sulphite and 2 ml of holdback carrier solution B; then add, with vigorous stirring, excess of trimethylphenylammonium iodide solution. Set aside for a few minutes, spin in a centrifuge, reject the supernatant solution to active waste, and wash the precipitate once with trimethylphenylammonium iodide wash solution. At this stage, the activity is generally down to tracer level.

Boil the precipitate with 5 ml of perchloric - sulphuric acid mixture. Iodine is evolved, and then the solution slowly clears; if necessary, add further small volumes of the acid mixture until a pale yellow or colourless solution results. Cool, dilute until no further heat is evolved, and cool again. Add 2 ml of holdback carrier solution C, 8 ml of 20 per cent. potassium bromide solution and 16 ml of brucine sulphate solution. Stir vigorously to coagulate the precipitate, set aside for at least 10 minutes, spin in the centrifuge, and reject the supernatant solution. Wash the precipitate once with brucine wash solution, and boil it with small volumes of the acid mixture to obtain a pale solution. Cool, dilute a little, add 1 ml of iron-scavenge solution, and stand the tube in ice - water mixture. Cautiously add ammonia solution, sp.gr. 0·880, until an excess is present, boil, filter, and wash the residue with hot ammonia solution. Stir into the filtrate 0·1 g of potassium iodide, and add 1 ml of a silver nitrate solution containing 10 mg of silver per ml. Boil to coagulate the silver iodide, filter, and wash the precipitate with ammonia solution. Dissolve 1 g each of Rochelle salt and sodium sulphite in the filtrate, cool, and add concentrated sulphuric acid dropwise until the solution is yellow and acid. Add 2 g of potassium iodide and 2 ml of holdback carrier solution B, and dilute to 30 ml with water. Add 5 ml of 5,6-benzoquinoline solution, with thorough stirring, and warm, if necessary, to initiate coagulation. Allow the precipitate to settle, add a surface layer of ethanol to repel the solid, and spin in the centrifuge. Discard the supernatant solution, and wash the precipitate once with 5,6-benzoquinoline wash solution; stir well to dissolve any precipitated reagent, and add ethanol before centrifugation. Dissolve the residue in the minimum of acid mixture by boiling, and cool the clear solution. Dilute a little, and add 2 ml of holdback carrier solution C and some decolorising charcoal. Boil for 1 minute, allow to cool to about 50° C, add 5 ml of 20 per cent. potassium bromide solution, and filter. To the cooled filtrate, which should be nearly colourless, add 10 ml of brucine sulphate solution, stir to initiate precipitation, and set aside for at least 20 minutes.

Stir, spin in the centrifuge, and reject the supernatant solution. Wash the white precipitate twice with brucine wash solution and then three times with a mixture of ethanol and diethyl ether (1 + 5), breaking up the lumps of solid. Suspend the "brucinate" in a little pure diethyl ether, transfer to a tared aluminium counting tray, distribute the solid evenly, and dry under a heat lamp. Allow to cool, weigh, and count next day in a beta-counter.

PROCEDURE FOR STANDARDS—

Prepare 50-ml portions of standard solutions containing 0·01 to 1·0 μg of cadmium as described under "Preparation of Standards and Samples for Irriadation." Evaporate aliquots (containing 0·1 μg of cadmium) of the concentrated standards and the total volumes of the less concentrated ones to 5 ml. To each solution add 10 mg of cadmium carrier, as sulphate solution, and then 1 ml of iron-scavenge solution and excess of ammonia solution. Filter, wash the residue, add the washings to the filtrate, and stir in 0·1 g of potassium iodide. Add 1 ml of the silver nitrate solution, boil to coagulate the silver iodide, and filter or spin in the centrifuge. Wash the residue once with dilute ammonia solution. Dissolve in the filtrate 1 g each of Rochelle salt and sodium sulphite, cool, and acidify with concentrated sulphuric acid. Add 1 g of potassium iodide, 2 ml of holdback carrier solution B and excess of trimethylphenylammonium iodide solution, with vigorous stirring.

Allow the precipitate to settle, spin in the centrifuge, discard the supernatant solution, and wash the precipitate once with trimethylphenylammonium iodide wash solution. Dissolve it by boiling in the minimum amount of perchloric - sulphuric acid mixture, cool, dilute a little, and add 2 ml of holdback carrier solution C and 0·5 g of potassium chloride. Stir, filter, add 5 ml of 20 per cent. potassium bromide solution to the filtrate, thoroughly cool, and then add 10 ml of brucine sulphate solution. Stir well, set aside, wash the precipitate, and mount it for counting as described for samples of rock.

TESTS FOR RADIOCHEMICAL PURITY—

The simplest way to check the radiochemical purity of the sources is to follow the decay of the activity from 24 hours after preparation for about a week. Since the sources prepared from an average rock give only 50 to 100 counts per minute at the outset, the activity usually merges into the background after this period. For the same reason, it is not usually convenient to obtain absorption curves, and hence the maximum beta-energy, with sources from rock samples. When sources prepared from standards, together with aluminium absorbers, are used, maximum beta-energy occurs at about 460 mg per sq. cm, which corresponds closely to the published value of 1·1 MeV for ^{115}Cd.

The observed half-life is usually 56 to 57 hours, slightly longer than the accepted value for ^{115}Cd. The difference is ascribed to the presence of a small amount of ^{115m}Cd (half-life 43 days); since this is present in both samples and standards, which contain roughly the same weight of cadmium, it does not affect the validity of the comparison between the two. The small "tail" observed in the absorption measurements is attributed to the same cause (for ^{115m}Cd, maximum beta-energy is 1·6 MeV, equivalent to 740 mg per sq. cm of aluminium).

RESULTS AND DISCUSSION OF THE METHOD

The precision of the proposed method may be judged from the results in Table II for a series of rocks from the Skaergaard Intrusion, East Greenland.

TABLE II

CADMIUM CONTENTS FOUND IN SKAERGAARD ROCKS

Sample No.	Type of rock	Cadmium content found, p.p.m.
E.G. 5259 ..	Transgressive acid granophyre	0·57, 0·59, 0·54 (mean 0·57)
E.G. 4489 ..	Transgressive acid granophyre	0·44, 0·44 (mean 0·44)
E.G. 4332 ..	Basic hedenbergite granophyre	0·27, 0·28 (mean 0·28)
E.G. 4328 ..	Fayalite ferrogabbro, purple band	0·28, 0·27 (mean 0·28)
E.G. 5196 ..	Melanocratic ferrogabbro	0·11, 0·12 (mean 0·12)
E.G. 5181 ..	Average rock	0·38, 0·43 (mean 0·40)
E.G. 5321 ..	Plagioclase cumulite	0·34, 0·32 (mean 0·33)
E.G. 5052 ..	Middle gabbro (olivine-free)	0·08, 0·09 (mean 0·09)
E.G. 5087 ..	Lower olivine gabbro	0·20, 0·19 (mean 0·20)
E.G. 5086 ..	Lower olivine gabbro	0·08, 0·12 (mean 0·10)
E.G. 4526 ..	Gabbro picrite	0·08, 0·09 (mean 0·09)
E.G. 4443 ..	Border group	0·11
E.G. 4507 ..	Chilled marginal gabbro (initial magma)	0·13, 0·11, 0·13, 0·13 (mean 0·13)

The accuracy of our results can only be assessed for the standard rocks G-1 and W-1, for which independent analyses are available. The results in Table III show that agreement

with other methods is satisfactory for G-1, but not W-1, and, although we have endeavoured to eliminate potential sources of systematic error from our method, further independent determinations are clearly required.

TABLE III

CADMIUM FOUND IN STANDARD ROCKS BY VARIOUS METHODS

Method	Reference No.*	Cadmium found in— granite G-1, p.p.m.	Cadmium found in— diabase W-1, p.p.m.
Neutron activation (proposed procedure)	—	0·054, 0·056, 0·066 (mean 0·06)	0·30, 0·33, 0·34, 0·34 (mean 0·33)
Anion-exchange enrichment and spectrography	23	—	0·07, 0·09 (mean 0·08)
Square-wave polarography	7	0·06, 0·08, 0·09, 0·10 (mean 0·08)	0·24, 0·27, 0·33, 0·35 (mean 0·30)

* See reference list below.

The proposed procedure appears to be serviceable, but is probably capable of refinement. It may be possible with some samples to omit certain of the precipitation steps, so increasing the chemical yield (at present about 25 per cent.) and the sensitivity of the method. When carried out as described above, analysis of twelve samples of rock, with four standards, takes 4 days from the return of the irradiated material.

We thank Mr. A. A. Smales, U.K. Atomic Energy Research Establishment, Harwell, for suggesting the determination of cadmium as a problem in radioactivation analysis and Professor L. R. Wager for making available the Skaergaard material and for his interest in the work. The necessary irradiations in the Harwell pile BEPO were arranged through the Isotope Division of the Atomic Energy Research Establishment.

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The Determination of Tin in Pure Iron, Mild Steel and Certain Low-alloy Steels by Cathode-ray Polarography

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A method is described in which a linear-sweep cathode-ray polarograph is used for determining tin in pure iron and mild steel. Tin is separated by co-precipitation with manganese dioxide and is determined in a base electrolyte containing peptone and ascorbic acid in 5 M hydrochloric acid. Molybdenum interferes, but, provided that the molybdenum content of the sample does not exceed 0·5 per cent., a correction can be made, so that the method may also be used for the analysis of low-alloy steels. The method is suitable for tin contents down to 0·001 per cent., and a standard deviation of 0·001 per cent. is claimed at the 0·02 per cent. level.

A British Standard method for determining tin in iron and steel has been published.[1] After isolation of the sulphide with molybdenum sulphide as carrier, tin is reduced with metallic aluminium in the presence of an antimony salt and is then titrated with potassium iodate solution. For irons, carbon steels and certain low-alloy steels, the sulphide separation is unnecessary and the filtered solution can be titrated immediately. This method has been shown[2] to be suitable for tin contents in the range 0·01 to 0·25 per cent., and the reproducibility is stated to be within $\pm 0{\cdot}003$ per cent. when 0·05 per cent. of tin is present. A more precise method was required for the analysis of pure iron and mild steel containing 0·001 to 0·02 per cent. of tin, and it was considered that a polarographic approach might offer increased accuracy and sensitivity at these levels.

Ferrett and Milner[3] have described an attractive direct method for determining tin in steel by square-wave polarography and claim that it is suitable for tin contents as low as 0·0005 per cent.; chemical separations are unnecessary, and the valency state of the iron is unimportant. As base electrolyte, these workers used 5 M hydrochloric acid in preference to the 1 M hydrochloric acid - 4 M ammonium chloride mixture recommended by Lingane.[4] Trials with this method in conjunction with a cathode-ray polarograph were disappointing; instrumental sensitivity for tin reductions did not approach that of the square-wave polarograph, and application of the method was limited to steels containing more than 0·01 per cent. of tin, with a probable standard deviation of 0·01 per cent. Some form of separation was therefore necessary for determining tin contents less than 0·01 per cent.

As well as by precipitation as sulphide, tin can be separated from iron by precipitation as metastannic acid or distillation as chloride or bromide; all these techniques have been used as preliminaries to the polarographic determination of tin in steel. Allsopp and Damerell[5] described a method in which tin was co-precipitated with molybdenum sulphide and the reduction of stannic tin was recorded in Lingane's base electrolyte. Mahr and Waffenschmidt[6] distilled tin as tetrachloride by passing an azeotropic mixture of steam and hydrogen chloride through a solution of the sample in perchloric acid. An alternative separation procedure for alloy steel has been based on the method described by Kallmann, Liu and Oberthin,[7] in which a solution of the sample in sulphuric acid is distilled with a mixture of steam and hydrogen bromide; tin is co-precipitated from the distillate with aluminium hydroxide, and the wave for stannic tin is measured in 5 M hydrochloric acid. Co-precipitation of tin as metastannic acid with manganese dioxide from a solution of the sample in dilute nitric acid is a well established technique in non-ferrous analysis and has found particular application in the formulation of A.S.T.M. methods for analysing pig lead[8] and special brasses and bronzes.[9] As well as tin, arsenic and antimony are quantitatively precipitated, and iron, molybdenum, tungsten and bismuth are precipitated in part.

Gotô, Ikeda and Watanabe[10] first demonstrated the practical use of this separation combined with a polarographic finish for the analysis of steel. After separation, tin was reduced with metallic aluminium and was measured in Lingane's base electrolyte; antimony

was also co-precipitated and was measured in a sulphuric acid base electrolyte. Brháček later used powdered iron as reductant,[11] and Rooney used the same technique for separating antimony from cast iron.[12]

Of the three separation procedures considered, co-precipitation with manganese dioxide is possibly the simplest and most suited to repetitive analyses. In this paper, a polarographic method is described for determining tin in pure iron, mild steel and certain low-alloy steels after preliminary separation of the tin by a co-precipitation method closely similar to that described by Rooney.[12]

Experimental

Separation of tin—

Tests indicated that co-precipitation of tin with manganese dioxide was practically quantitative over a fairly wide range of concentrations of free acid. It seemed desirable, however, to adhere as closely as possible to conditions found by other workers to be suitable for the co-precipitation of antimony, so that, if necessary, this element could be determined at the same time. Babko and Shtokalo[13] found that co-precipitation of antimony was complete in the pH range 1 to 7; this was confirmed by Rooney,[12] who pointed out that, in order to obtain a pH in this range, the minimum amount of acid must be used to dissolve the sample and care must be taken to prevent loss of acid during solution so as to avoid hydrolysis of ferric iron. Trials with Gotô, Ikeda and Watanabe's procedure[10] produced precipitates heavily contaminated with iron and difficult to filter. Rooney[12] incorporated preliminary treatment with potassium permanganate to oxidise carbonaceous matter; the excess of permanganate was removed with hydrogen peroxide, and a controlled addition of permanganate was then made to precipitate manganese dioxide. This technique was more satisfactory, provided that the volume of solvent acid recommended was increased by 10 to 15 per cent. to prevent hydrolysis of iron salts. The degree to which iron was co-precipitated was largely dependent on the concentration of acid and the amount of permanganate used in the initial oxidation. Contamination by iron was considerably decreased when the volume of permanganate solution was kept to a minimum. The presence of manganese salts, as suggested by Gotô, Ikeda and Watanabe,[10] improved the coagulation of the precipitate, especially when it contained iron.

Tests on samples of steel produced precipitates that could easily be separated on Whatman No. 541 filter-papers. Samples containing molybdenum produced precipitates that did not settle rapidly; for such samples it was necessary to pass the solution through the filter-paper two or three times to obtain a clear filtrate.

Polarographic measurement—

Most previous workers reduced tin to the bivalent state after solution of the manganese dioxide precipitate in hydrochloric acid and hydrogen peroxide. It seemed more convenient, however, to determine tin in the stannic state, thereby avoiding the difficulties encountered in the reduction and in maintaining the element in the stannous state during polarography. The polarographic behaviour of tin in the presence of chloride has been investigated by Lingane.[4,14] Stannic tin produces an ill defined wave in 1 M hydrochloric acid at −0·47 volt, with a small pre-wave beginning at about −0·05 volt. As the concentration of chloride is increased, the waves are resolved into two well defined steps having half-wave potentials at −0·25 and −0·52 volt in a base electrolyte consisting of 4 M ammonium chloride and 1 M hydrochloric acid. The first wave involves a two-electron reduction of the hexachlorostannate ion to tetrachlorostannate and is irreversible; the second wave is produced by the reduction of tetrachlorostannate to the metal. Ferrett and Milner[3] reported inconsistent results for tin when Lingane's base electrolyte was used; they used 5 M hydrochloric acid in order to obtain maximum definition. With use of a cathode-ray polarograph, I have confirmed this observation and found that peak heights are consistent for concentrations of hydrochloric acid in the range 3 to 6 M. Irreversible reductions produce characteristically rounded and rather drawn-out waves on a cathode-ray polarogram, and the second wave, which has a peak potential of −0·48 volt against the mercury-pool anode, was therefore chosen for quantitative measurement. The diffusion-current plateau of the first wave (peak potential at about −0·2 volt) provides a horizontal or nearly horizontal base line for measuring the height of the main wave for stannic tin. Antimony, if present, is reduced at a peak potential of −0·15 volt, and its wave is therefore superimposed on the first tin wave; it does not interfere with the second tin wave.

At first, waves were ill defined and had sloping base lines (see Fig. 1), but the addition of 5 mg of peptone to the contents of the polarographic cell after de-oxygenation improved the definition considerably. The presence of a small unidentified wave at the foot of the tin wave still caused difficulties; however, when the solution was evaporated until fumes were evolved and fuming was maintained for a few minutes, this unidentified wave disappeared (see Fig. 2) and measurement of the peak height became much easier. Tests showed that, if fuming was continued for 5 minutes, there was some danger of loss of tin, presumably by volatilisation, and for this reason heating to fumes for 2 minutes is stipulated in the proposed procedure.

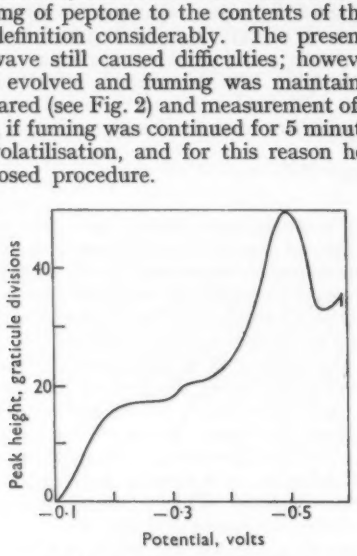

Fig. 1. Typical polarogram for tin (0·02 per cent.) in B.C.S. No. 272 before inclusion of heating to fumes and addition of peptone

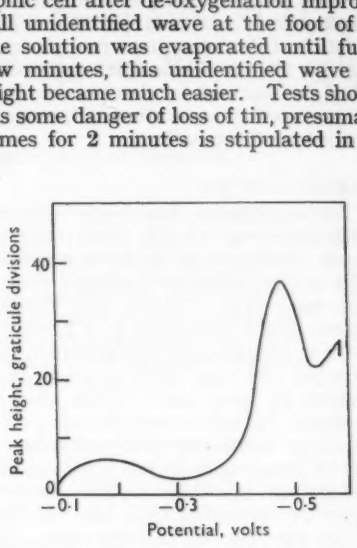

Fig. 2. Polarogram for tin in B.C.S. No. 272 after heating to fumes and addition of peptone

The proposed method was tested by adding different amounts of a tin solution to 1-g samples of pure iron dissolved in 35 ml of dilute nitric acid (1 + 4) without heating. Some difficulty was experienced in preparing a standard tin solution in dilute nitric acid, but a satisfactory technique was established by dissolving AnalaR tin in dilute nitric acid (1 + 4) without heating. The tin dissolves completely in about 2 hours, and the solution is stable for several hours, provided that the room temperature does not exceed about 20° C. The percentage of tin recovered was determined by comparison with a calibration graph plotted from the results obtained with solutions of pure tin. The results are shown in Table I and indicate that the mean recovery is 97 per cent. in the range 0·01 to 0·10 per cent. of tin and between 90 and 110 per cent. for tin contents below 0·01 per cent. This is considered to be satisfactory for a method depending on co-precipitation; it seems probable that complete recovery could be obtained by a second precipitation from the filtrate, but this would considerably increase the time required for a determination. In order that the method of calibration should be representative of the method of test, the calibration procedure in the proposed method is based on adding tin to a solution of pure iron. Similar tests were carried out on 5-g samples of pure iron dissolved in 135 ml of solvent acid, and the results are also shown in Table I. The mean recovery was 87 per cent., which can hardly be considered satisfactory for accurate determination of tin at the 0·01 per cent. level. On the other hand, use of the larger weight of sample permitted the extension of the method to a lower limit of 0·0002 per cent. of tin, and poor recovery may therefore be balanced by improved sensitivity in the range, say, 0·0002 to 0·002 per cent. of tin.

Method

Reagents—

All reagents should be of the highest grade of purity obtainable.

Standard tin solution—Transfer exactly 50 mg of AnalaR tin metal to a 500-ml calibrated flask, add 250 ml of dilute nitric acid (1 + 4), and allow the metal to dissolve; maintain the temperature below 20° C. Dilute to the mark with dilute nitric acid (1 + 4), and use immediately. One millilitre of this solution is equivalent to 100 μg of tin (0·01 per cent. of tin in a 1-g sample or 0·002 per cent. in a 5-g sample).

PROCEDURE—

Transfer 1 g of sample (see Note) to a 400-ml conical beaker, add 35 ml of dilute nitric acid (1 + 4), and allow to dissolve without heating. Add 150 ml of water and 10 ml of a 10 per cent. w/v solution of manganese sulphate, insert a boiling rod, and heat to boiling-point on a hot-plate. Oxidise the solution with a 1 per cent. w/v solution of potassium permanganate, added dropwise. When the solution has attained a deep-purple colour, add 5 drops of permanganate solution in excess, and boil gently for 3 to 5 minutes. Reduce the solution by adding 20-volume hydrogen peroxide dropwise, and boil to remove excess of peroxide. Dilute the solution to 200 ml, if necessary, bring to the boil, and add 2 ml of the potassium permanganate solution. Boil gently for 15 minutes, remove from the hot-plate, allow the precipitate to settle, and filter the solution through a fluted Whatman No. 541 filter-paper into a clean beaker. (If the sample contains molybdenum, the precipitate settles slowly, and several filtrations may be necessary before a clear filtrate is obtained; set solutions from such samples aside for about 30 minutes before filtration.) The filtrate should be

TABLE I

RECOVERY OF TIN ADDED TO SOLUTIONS OF PURE IRON

Tin added, %	Tin recovered from— 1-g sample of iron, %	5-g sample of iron, %
0·0005	—	0·0004, 0·0004
0·001	0·001, 0·001	0·0010, 0·0008
0·002	0·002, 0·002	—
0·004	—	0·0034, 0·0036
0·005	0·0045, 0·0055	—
0·01	0·0095, 0·0095	0·0080, 0·0080
	0·0095, 0·0105	—
	0·0095, 0·0095	—
0·02	—	0·0168, 0·0170
0·10	0·096, 0·098	—
	0·095, 0·097	—
	0·098, 0·099	—

perfectly clear; if it is not, re-filter it through the same filter-paper until a clear filtrate is obtained. Wash the precipitate thoroughly with hot water. Place the funnel containing the filter-paper in the neck of the original beaker, pierce a hole in the paper with a pointed glass rod, and wash the precipitate into the beaker with hot water and dropwise additions of diluted hydrochloric acid (1 + 1) and 20-volume hydrogen peroxide. To the solution add 5 ml of nitric acid and 10 ml of diluted sulphuric acid (1 + 1), evaporate until fumes are evolved, and continue to fume for 2 minutes. Cool, add 10 ml of diluted hydrochloric acid (1 + 1), heat to boiling-point, and oxidise with 2 or 3 drops of a saturated solution of potassium chlorate. Concentrate the solution to about 10 ml, add 10 ml of water and 20 ml of hydrochloric acid, sp.gr. 1·18, cool, and dilute to 50 ml in a calibrated flask. Add 1 g of ascorbic acid, and shake until solution is complete.

Transfer 2 to 3 ml of the solution to the cell of a cathode-ray polarograph (Southern Analytical Ltd.), remove oxygen by passing nitrogen for 5 to 10 minutes, add about 5 mg of peptone, and pass nitrogen until solution is complete. Measure the peak height of the second tin wave; use a start potential of −0·10 volt. (If the sample contains tungsten, a wave will be visible at about −0·6 volt, and it may be necessary to use the derivative circuit to resolve the tin wave; for such solutions, re-set the start potential at −0·30 volt.) If the sample contains molybdenum, a correction factor must be applied; multiply the apparent tin content of such a sample by the factor—

$$\frac{100}{100 - (\text{Molybdenum content, \%} \times 50)}$$

to give the true tin content. If the derivative circuit is used, the apparent tin content must be multiplied by the factor—

$$\frac{100}{100 - (\text{Molybdenum content, \%} \times 25)}$$

For other types of polarograph, the extent to which molybdenum interferes should be established as described under "Interfering Elements."

With each batch of samples, examine a reagent blank and a standard steel as a check on the calibration graph. The peak current of the reagent blank is normally 0·02 to 0·06 μA.

NOTE—The sensitivity and lower limit of detection can be improved by using a 5-g sample dissolved in 135 ml of 20 per cent. v/v nitric acid. Separation is then only about 87 per cent. complete, and it is therefore essential to plot a separate calibration graph based on a 5-g sample.

CALIBRATION—

Prepare solutions by dissolving 1-g portions of pure iron having a low tin content in dilute nitric acid (1 + 4). To these solutions add amounts of standard tin solution suitable for the construction of two calibration graphs to cover the ranges 0·001 to 0·01 per cent. and 0·01 to 0·10 per cent. of tin. The volume of dilute acid used for dissolving the iron should be decreased by an amount equal to the volume of tin solution added, so that the total volume of final solution is always 35 ml. Treat these solutions as described under "Procedure."

INTERFERING ELEMENTS

IRON—

If the amount of co-precipitated iron exceeds a few milligrams, reduction of ferric iron at zero potential gives rise to a large standing current, which cannot be fully "backed-off." This can be avoided by reducing the iron to the ferrous state with ascorbic acid, but even then, excessive amounts of ferrous iron tend to produce a sloping base line to the tin wave and may cause difficulties in polarographic measurement at high instrumental sensitivity.

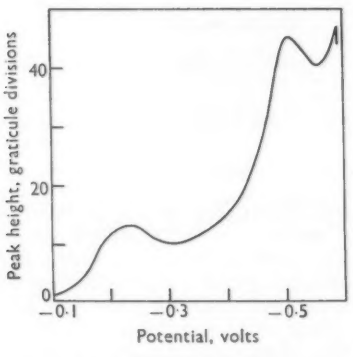

Fig. 3. Effect of molybdenum on tin wave. Sample contained 0·01 per cent. of tin and 0·5 per cent. of molybdenum

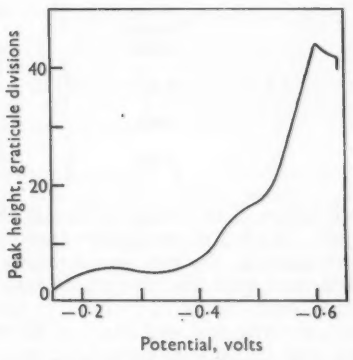

Fig. 4. Effect of tungsten on tin wave. Sample (B.C.S. No. 277) contained 0·003 per cent. of tin and 0·12 per cent. of tungsten

ARSENIC—

There is no arsenic wave when the value selected for the start potential is more negative than −0·05 volt, but the presence of arsenic distorts the tin wave and causes high results; the extent of interference is dependent on the start potential. To prevent this interference, arsenic is oxidised to the quinquivalent state with potassium chlorate after the solution has been fumed; quinquivalent arsenic is not reduced at the mercury electrode.

MOLYBDENUM—

In concentrated hydrochloric acid, molybdenum is reduced at about −0·25 volt, producing a small rounded wave; its presence leads to base-line difficulties, which are particularly serious when the concentration of tin is low and the ratio of molybdenum to tin is high. Molybdenum distorts the shape of the first tin wave, which becomes more rounded and less well defined, and the slope of the base line of the second wave progressively increases with the concentration of molybdenum. This is indicated in Fig. 3, which shows the effect of 0·5 per cent. of molybdenum in a sample containing 0·01 per cent. of tin. To determine the effect of molybdenum on the peak height of the tin wave, various amounts of the element were added to solutions containing 1 g of pure iron and four different levels of tin. The

recoveries of the tin are shown in Table II and indicate that molybdenum exerts negative interference. At each level of tin, increasing amounts of molybdenum decrease the height of the tin wave by a value approximately proportional to the amount of molybdenum added, *i.e.*, for each 0·1 per cent. of molybdenum, the peak height is decreased by 5 per cent. A correction can therefore be applied to the apparent tin content to correct for interference from molybdenum, provided that the molybdenum content does not exceed 0·5 per cent.; at higher contents of molybdenum interference is inconsistent and difficult to measure. A similar experiment, in which the derivative circuit was used, indicated that the peak height is decreased by approximately 2·5 per cent. for each 0·1 per cent. of molybdenum, but this decrease in interference is offset by a considerable reduction in sensitivity.

TABLE II

EFFECT OF MOLYBDENUM ON RECOVERY OF TIN ADDED TO 1-g SAMPLES OF PURE IRON

Molybdenum added, %	Tin added, %	Tin found, %	Mean recovery, %
0·1	0·02	0·019, 0·019	95
	0·10	0·092, 0·094	93
0·2	0·004	0·0035, 0·004	94
	0·01	0·009, 0·0085	88
	0·02	0·018, 0·018	90
	0·10	0·086, 0·088	87
0·4	0·004	0·003, 0·0035	81
	0·01	0·0075, 0·008	78
	0·02	0·0165, 0·017	84
	0·10	0·080, 0·082	81
0·5	0·004	0·003, 0·003	75
	0·01	0·008, 0·007	75
	0·02	0·016, 0·016	80
	0·10	0·076, 0·078	77

TUNGSTEN—

Tungsten is reduced at about −0·6 volt and produces a rather poorly defined wave, which tends to merge with the tin wave when the ratio of tungsten to tin approaches 10 to 1; Fig. 4 shows the effect of tungsten when the ratio of tungsten to tin was 40 to 1. If the ratio of 10 to 1 is exceeded, the derivative circuit must be used to resolve the tin wave. With this circuit it has been found possible to determine 0·01 per cent. of tin in a sample known to contain 1 per cent. of tungsten.

RESULTS

The proposed method was applied to three British Chemical Standard samples of pure iron and one sample of B.I.S.R.A. vacuum-melted pure iron (AH iron). The results are shown in Table III; at the level of 0·002 per cent. of tin, the standard deviation is 0·0005 per cent. The tin contents of three of these irons were also determined on 5-g samples; the results, expressed to the nearest 0·0002 per cent., were—

Sample	B.C.S. No. 149/1	B.C.S. No. 260/1	AH iron
Tin content found, %	0·0014, 0·0014	0·0018, 0·0018	0·0018, 0·0022

and the standard deviation was estimated to be about 0·0002 per cent. of tin.

When it had been established that the method was suitable for analysing pure iron, a series of British Chemical Standard mild steels, containing alloying elements up to a maximum of 0·3 per cent., and two low-alloy steels containing molybdenum were analysed; the results are shown in Table IV. The peak heights for some of these samples were also measured by means of the derivative circuit. It is evident from Table IV that there is good agreement between results by the proposed and established procedures, with the possible exception of B.C.S. No. 276. It was not possible to measure the peak height for B.C.S. No. 277 with the direct circuit because of interference from tungsten, but resolution was perfect with the derivative circuit. The standard deviation is 0·001 per cent. at the 0·02 per cent. level and 0·005 per cent. at the 0·10 per cent. level.

Table III

Tin contents found by proposed method in 1-g samples of iron

Each result is expressed to the nearest 0·0005 per cent.

Sample	B.C.S. certificate value for tin, %	Tin content found, %	Mean, %
B.C.S. No. 149	<0·002	{0·0005, 0·001 / 0·0005, 0·001}	0·001
B.C.S. No. 149/1	0·002	{0·0025, 0·002, 0·0015 / 0·002, 0·0025, 0·0015}	0·002
B.C.S. No. 260/1	<0·002	{0·0015, 0·0015, 0·0015 / 0·002, 0·0025, 0·002}	0·002
AH iron	—	{0·001, 0·001, 0·001 / 0·001, 0·002, 0·002}	0·0015

Table IV

Tin contents found by proposed method in 1-g samples of mild and low-alloy steels

Certificate values for tin in B.C.S. Nos. 271, 272, 273, 274, 275, 276 and 277 are quoted to the nearest 0·005 per cent. Results for tin in the range 0·02 to 0·10 per cent. are expressed to the nearest 0·001 per cent. and those below 0·02 per cent. to the nearest 0·0005 per cent.

Sample	B.C.S. certificate value for—			No. of determinations	Tin content found—	
	tin, %	molybdenum, %	tungsten, %		(direct circuit), %	(derivative circuit), %
B.C.S. No. 271 ..	0·110	0·19	0·02	6	0·110	—
B.C.S. No. 272 ..	0·020	0·17	0·01	3	0·020	0·019
B.C.S. No. 273 ..	0·065	0·05	0·28	3	0·068	—
B.C.S. No. 274 ..	0·020	0·07	0·04	3	0·025	0·023
B.C.S. No. 275 ..	0·040	0·10	0·05	3	0·044	—
B.C.S. No. 276 ..	0·010	<0·01	0·20	6	0·017	0·018
B.C.S. No. 277 ..	<0·005	0·02	0·12	3	—	0·003
B.C.S. No. 239/1 ..	0·06	0·03	—	3	0·049	—
B.C.S. No. 239/2 ..	0·024	0·03	—	3	0·022	0·020
B.C.S. No. 218/2 ..	0·035	0·03	—	3	0·033	—
B.C.S. No. 219/2 ..	~0·025	0·43	0·19	3	0·026	0·023
B.C.S. No. 256 ..	~0·013	0·54	—	6	0·012	0·012

Conclusions

Separation of tin by co-precipitation on manganese dioxide is a suitable preliminary to the determination of this element by cathode-ray polarography. The method offers considerable improvement in sensitivity and accuracy over the British Standard volumetric procedure and was primarily designed for determining 0·001 to 0·02 per cent. of tin in pure iron and mild steel. Higher contents of tin can be determined in samples soluble in dilute nitric acid, provided that the molybdenum content does not exceed 0·5 per cent. Tungsten interferes with the measurement of the tin wave when the tungsten content is more than ten times the tin content. If this ratio is exceeded, it is possible to obtain reasonably accurate results by using the derivative circuit of the instrument to measure the tin wave.

The sensitivity of the method may be further improved by using a larger weight of sample; the theoretical limit of detection is extended down to 0·0002 per cent. of tin in a 5-g sample. Poor recovery with large weights of sample is a disadvantage, but this is balanced by an improvement in sensitivity.

Previous workers[10,11,12] have shown that co-precipitation on manganese dioxide is also suitable for isolating antimony from iron and steel, and it should therefore be possible to determine this element in an aliquot from the solution after separation. Base electrolytes suitable for the polarographic determination of antimony have been described.[10,11]

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Polarographic Determination of Antimony in Refined Lead*

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A polarographic method has been developed for determining antimony in refined lead. The supporting electrolyte used is N sulphuric acid. Copper, iron and bismuth do not interfere and there is no interference from tin even if present in amounts equal to the antimony. However, results for antimony are lower if tin is present in higher ratios. The method can be used for determining as little as 10 p.p.m. of antimony on a 1-g sample of lead; the error is within ±5 per cent. in the concentration range above 25 p.p.m., but is greater in the lower range.

THE concentration of antimony in refined lead is extremely low, and, according to Green,[1] if more than 100 p.p.m. are present, it seriously affects the rate of oxidation of the lead. Von Bayerl[2] and Kalousek[3] studied polarographically the reduction of antimony in hydrochloric acid medium and found that the results were only qualitative. Kraus and Novak[4] pointed out that the variations observed were due to changing acid concentration; Hourigan[5] found that variations were also due to differences in lead concentrations. Hourigan and Robinson[6] determined antimony in antimony - lead alloys and found that lead up to a concentration of 0·03 M could be tolerated. Besides copper, iron and equal amounts of bismuth interfere in this method, which is obviously not suitable for determining traces of antimony in refined lead.

In a paper on the determination of traces of impurities in refined lead, Zotta[7] suggested the use of sulphuric acid - nitric acid medium as supporting electrolyte. Under the conditions she described it was observed that, owing to solution of mercury, good waves were not obtained. Moreover, Kolthoff and Lingane[8] had suggested that on dissolving lead in nitric acid Sb^{3+} may be oxidised to Sb^{5+} and, in presence of tin, it may remain undissolved as antimony pentoxide.

The basis of the method described here is solution of the sample in nitric acid, removal of lead as lead sulphate, evaporation of an aliquot of the solution to remove excess of nitric acid and then determining the antimony with N sulphuric acid as supporting electrolyte.

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Experimental

A 0·01 M solution of antimony trichloride was prepared from the analytical-reagent grade salt, the acidity being kept at 5 per cent., and the solution was standardised iodimetrically. Two solutions, one containing 100 and the other 10 μg of antimony per ml, were prepared from this stock solution. Solutions of copper sulphate, bismuth nitrate, ferric chloride and stannous chloride containing 100 μg of the metal per ml were prepared from the analytical-reagent grade salts and standardised by conventional methods. Laboratory-reagent grade sulphuric acid was used.

A Du Bellay polarograph was used. Temperature was maintained constant at 30° $\pm$ 0·1° C by an electronically controlled thermostat. The rate of outflow (m) of the capillary was 1·217 mg per second and the drop rate (t) was 4·4 seconds. The mercury pool was used as anode.

Different amounts of antimony (5 to 600 μg), as trichloride, were placed in beakers and 0·4 ml of concentrated sulphuric acid was added to each. The solutions were evaporated until fumes were evolved, and then polarograms were recorded with 3·0 ml of N sulphuric acid as supporting electrolyte. In another set of experiments different amounts of antimony, as trichloride, were first treated with 5 ml of dilute nitric acid (1 + 4), and then the procedure described above was repeated.

Table I

Recovery of antimony from lead after treatment with nitric acid

A 1-g sample of lead was used for each test. All results have been corrected for the antimony (about 25 p.p.m.) present in the lead

Amount of antimony added, μg	Amount of antimony recovered, μg	Difference, %
10	9·1	−9
10	10·6	+6
25	25·0	0
25	25·2	+0·8
50	52·5	+5
50	51·2	+2·4
100	100·0	0
200	200·0	0
600	594·0	−1

Proportionality was observed for 5 to 600 μg of antimony and the results were not affected by the treatment with nitric acid. Recovery experiments for antimony were carried out on 1-g samples of lead to which different amounts of antimony, in solution, were added. The metal was dissolved in 5 ml of dilute nitric acid (1 + 4). The solution was transferred to a 25-ml flask, 1 ml of concentrated sulphuric acid was added to precipitate lead, and the solution was made up to 25 ml. From this, 10 ml were placed, by pipette, in a beaker, and evaporated to the appearance of fumes; 3·0 ml of N sulphuric acid were then added, and the wave for antimony was recorded. Results are shown in Table I.

The effect of tin was investigated by adding different amounts of tin, as stannous chloride, to the antimony solution and then carrying out the treatment with dilute nitric acid (1 + 4). Antimony was determined as described above and it was found that a ratio of tin to antimony of almost 2 to 1 could be tolerated. Recovery of antimony in presence of different amounts of tin, as stannous chloride, was tested on a 1·0-g sample of lead. The lead was dissolved by the nitric acid treatment; the results are shown in Table II.

The half-wave potentials of arsenic, antimony and bismuth in N sulphuric acid are reported as −0·7, −0·32 and −0·04 volt against the saturated-calomel electrode, respectively.[9] These elements were also taken with antimony and the recoveries were tested on 1·0-g samples of lead. The half-wave potential for arsenic is much higher than that for antimony and therefore it does not interfere. The results are shown in Table II; the original antimony content of the lead is corrected for.

Antimony in two samples of refined lead was determined by the method described, and the results of duplicate and triplicate determinations at two sensitivites of the instrument are shown in Table III.

Table II

Effect of different elements on the determination of antimony in lead

A 1-g sample of lead was used for each test

Amount of antimony added, µg	Impurity added	Amount of impurity added, µg	Amount of antimony recovered, µg	Mean recovery, %
100	Tin	100	100 100 100	100
100	Tin	200	87·15 87·15 88·90	87·73
100	Tin	300	71·4 71·4 72·7	71·83
100	Copper Iron Bismuth Tin	100 100 100 100	95·0 95·0 95·4	95·13

Table III

Determination of antimony in refined lead

Sample No.	Sensitivity × 10^{-9}, amps per mm	Antimony found, p.p.m.	Mean, p.p.m.
I	6·3	24·4, 22·7, 24·4	24·1
	4·0	25·0, 22·8, 25·0	
II	6·3	24·4, 24·4	24·6
	4·0	24·5, 25·0	

Discussion of the method

As it is difficult to dissolve the sample in hydrochloric acid, dilute nitric acid (1 + 4) is used. Zotta also used nitric acid, but, after precipitation of lead as lead sulphate, she suggested using the supernatant liquid for polarography. The supporting electrolyte was therefore a nitric acid - sulphuric acid mixture, in which it was found that the mercury from the drop as well as from the pool anode dissolved with the formation of mercuric nitrate. The mercuric nitrate so formed was reduced at the dropping electrode, thereby causing constant changes in height of the wave. Heating the test solution until fumes of sulphur trioxide were evolved removed nitric acid, and the wave could then be recorded in 3 ml of N sulphuric acid without any difficulty.

It is reported that nitric acid may oxidise antimony to antimony pentoxide, which makes solution impossible, especially if tin is present. Different amounts of antimony were added to 1·0-g samples of lead, and the recoveries show that antimony is not lost as the pentoxide. When equal amounts of tin and antimony were added to lead the recovery was 100 per cent. For a ratio of antimony to tin of 1 to 2 the recovery was 87 per cent. and was much less for higher ratios.

Bismuth, iron and copper in equal proportions do not interfere.

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The Complex of Vanadyl^{V} with Ethylenediaminetetra-acetic Acid

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From conductimetric measurements, the existence of a 1 to 1 complex of vanadyl^{V} ions and ethylenediaminetetra-acetic acid is established, and its application to volumetric analysis is suggested. The ratio of hydrogen ions to vanadium in the initial solution is critical and must exceed 5; the pH must be above 1·8. Conductimetric end-point detection is compared with a method in which xylenol orange is used as indicator.

Complexometric investigations of the vanadyl^{V} ion, VO_2^+, with phosphate, citrate, tartrate, oxalate and ethylenediaminetetra-acetic acid (EDTA) ions have revealed marked complexation around pH 2 with oxalate and EDTA, and an attempt was made to define the quantitative character of the VO_2^+ - EDTA complex[1] by the titration technique. It is obvious that the existence of this complex will, to a large extent, depend on the hydrogen-ion concentration, *i.e.*, the minimum acidity required for its quantitative formation from the vanadate ion[2]—

$$VO_3^- + 2H^+ \rightleftharpoons VO_2^+ + H_2O \quad .. \quad .. \quad .. \quad .. \quad (1)$$

whereas the maximum acidity will be determined by the general stability requirements of a metal - EDTA complex—

$$Me^{n+} + H_2Y^{2-} \rightleftharpoons (MeY)^{n-4} + 2H^+ \quad .. \quad .. \quad .. \quad (2)$$

With increasing acidity, this equilibrium will be displaced to the left and the stability of the complex decreased.[3]

In this investigation the ratio of hydrogen ions to vanadium was varied between 1 to 1 and 60 to 1 and the vanadium concentration between 0·015 and 0·009 gram-atom of vanadium per litre. Owing to a rather limited choice of low-pH complexometric indicators,[4,5,6] end-point determinations were in the first instance carried out conductimetrically. When the existence of the complex had been established the indicator method was tried, with xylenol orange as indicator.

Experimental

Reagents—

Ammonium metavanadate—Analytical-reagent grade.

Perchloric acid, 60 *per cent. w/w*—Analytical-reagent grade.

EDTA solution—A standard solution of the disodium salt of ethylenediaminetetra-acetic acid.

Xylenol orange solution—A 0·1 per cent. solution in dilute ethanol.

Preparation of stock vanadyl solution—

Dissolve 1·1699 g of ammonium metavanadate in approximately 400 ml of distilled water, heat to assist solution, add 60 ml of M perchloric acid, heat to boiling to ensure complete conversion of the polyvanadate to the pale-yellow vanadyl ion, and make up to 1 litre with distilled water. This stock solution is 0·01 M with respect to vanadium and 0·06 M in perchloric acid.

Alternatively, the vanadyl stock solution has been prepared by passing ammonium metavanadate solution through a column containing Zeo-Karb 225 resin[7] and receiving the solution of polyvanadic acid in perchloric acid of the requisite concentration. For conductimetric work the latter method has the advantage of eliminating ammonium ions. Its disadvantage is the need for subsequently determining vanadium, some of which is lost by adsorption on the resin.

Titration—

Transfer 5 ml of the stock vanadyl solution to a conical flask, dilute to the volume shown in Table III, p. 405, add 5 drops of xylenol orange solution,[8] and titrate in the cold with

0·01 M EDTA solution until the colour changes from red to pure yellow. Pyrocatechol violet[9] did not show a colour change.

For conductimetric titration (see Figs. 1 and 2), transfer 25 ml of the stock solution to a conductivity titration flask, dilute to about 200 ml with distilled water, and titrate at room temperature with 0·05 M EDTA solution to minimise dilution effects.

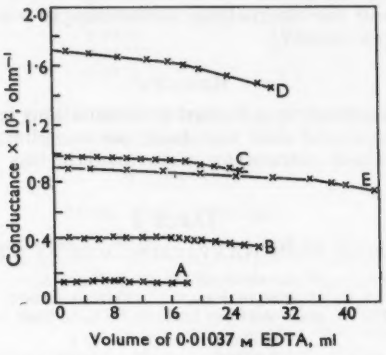

Fig. 1. Conductimetric titrations of solutions (200 ml) containing polyvanadic and perchloric acids (concentrations as shown in Table I) with 0·050 M EDTA at about 20° C

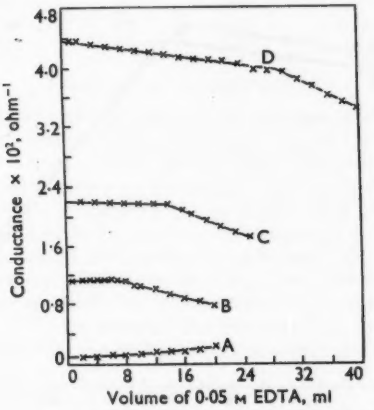

Fig. 2. Conductimetric titrations of solutions (200 ml) containing ammonium metavanadate and perchloric acid (concentrations as shown in Table II) with 0·01037 M EDTA at about 20° C

Accurate conductivity measurements (see Fig. 3) were made at 25° ± 0·001° C on solutions prepared individually with conductivity water. In each test 10 ml of vanadyl stock solution (polyvanadic acid *plus* perchloric acid), being 0·01845 M in vanadium and having a ratio of hydrogen ions to vanadium of 6·57 to 1, were transferred to a 50-ml calibrated flask. The required amount of 0·0104 M EDTA solution was then added, and the volume made up to 50 ml. This solution was transferred to a conductance cell, and, after it had been set aside for about 2 hours in a thermostatically controlled bath, its resistance was measured until constant readings were attained. These solutions were then retained for 7 days to attain equilibrium,[10] when their resistances were measured again.

EQUIPMENT—

Conductimetric titrations were carried out with a Cambridge conductivity bridge and dip-type electrodes. The titration flask was fitted with a stirrer. Accurate conductance measurements were made with a Jones and Bollinger type cell[11] kept at constant temperature in a Townson & Mercer S200 thermostatically controlled bath containing Puremore 210 light oil. The bridge consisted of Muirhead decade resistance boxes, the null-point was detected on a Cossor oscilloscope and the alternating-current supply was obtained from an Airmac oscillator at 1500 cycles per second.

RESULTS

Results based on conductimetric end-point determinations are shown in Tables I and II. In Table I, for which polyvanadic acid was used, the vanadium concentration was varied, whereas in Table II, for which ammonium metavanadate was used, it was kept constant (except for curve 2 E).

TABLE I

CONDUCTIMETRIC TECHNIQUE WITH POLYVANADIC ACID AS THE SOURCE OF VANADIUM

Curve	Concentration of vanadium, gram-atoms per litre	Concentration of hydrogen ions,* gram-ions per litre	Ratio of hydrogen ions to vanadium	Theoretical end-point, ml	Experimental end-point, ml
1A	0·00382	0·00382	1	15·28	—
1B	0·00184	0·01595	7·59	7·36	7·4
1C	0·00368	0·02808	7·59	14·72	14·0
1D	0·00736	0·05234	7·59	29·44	28·8

* Including protons obtained from ion-exchange column during formation of polyvanadic acid.

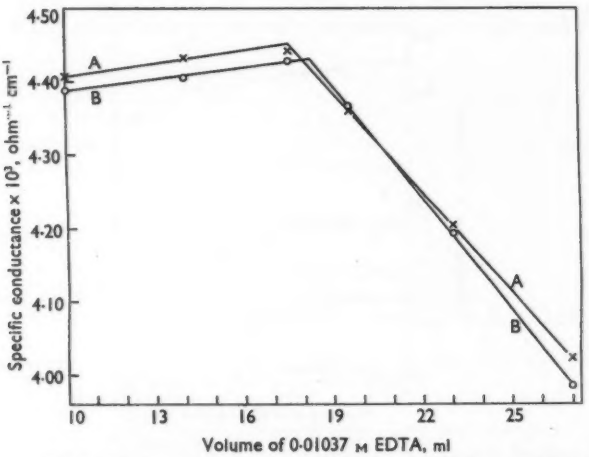

Fig. 3. Accurate conductance measurements at 25° ± 0·001° C of solutions containing polyvanadic and perchloric acids (0·00369 gram-atom of vanadium per litre; ratio of hydrogen ions to vanadium = 6·57) and different volumes of 0·01037 M EDTA (total volume made up to 50 ml): curve A, after 2 hours; curve B, after 7 days. Theoretical end-point at 17·79 ml

Conductimetric titration curves are shown in Figs. 1 and 2. As the breaks in these curves are in general somewhat ill-defined, only approximate experimental end-points are quoted in Tables I and II.

Fig. 3 shows accurate conductivity measurements on solutions 0·00369 M in vanadium and having a ratio of hydrogen ions to vanadium of 6·57 to 1. After 2 hours, the experimental end-point was reached on addition of 17·50 ml of standard EDTA solution, but, when set aside for 7 days, this break occurred at 18·20 ml (theoretical end-point at 17·79 ml).

TABLE II

CONDUCTIMETRIC TECHNIQUE WITH AMMONIUM METAVANADATE AS THE SOURCE OF VANADIUM

Curve	Concentration of vanadium, gram-atoms per litre	Concentration of hydrogen ions, gram-ions per litre	Ratio of hydrogen ions to vanadium	Theoretical end-point, ml	Experimental end-point, ml
2A	0·00088	0·00188	2·14	17·0	6·5
2B	0·00088	0·00555	6·3	17·0	16·8
2C	0·00088	0·0111	12·6	17·0	16·8
2D	0·00088	0·0175	20	17·0	17·5
2E	0·00178	0·0111	6·3	34·0	34·5

Table III shows titration results with xylenol orange as indicator and a stock vanadyl solution prepared from ammonium metavanadate.

TABLE III

INDICATOR TECHNIQUE

Total volume, ml	Concentration of vanadium, gram-atoms per litre	Concentration of hydrogen ions, gram-ions per litre	Ratio of hydrogen ions to vanadium	End-point: Theoretical, ml of 0·01 M EDTA (f = 0·986)	End-point: Experimental, ml of 0·01 M EDTA (f = 0·986)	Error, %	pH
(*a*) 100	0·0005	0·003	6	5·07	5·12	+1	—
(*b*) 100	0·0005	0·0055	11	5·07	5·09	+0·4	—
(*c*) 100	0·0005	0·013	26	5·07	5·10	+0·5	1·94
(*d*) 100	0·0005	0·018	36	5·07	4·8	−5	1·90
(*e*) 100	0·0005	0·023	46	5·07	—	—	1·80
(*f*) 10	0·005	0·03	6	5·07	5·05	−0·4	1·80
(*g*) 50	0·001	0·03	30	5·07	5·10	+0·6	—
(*h*) 100	0·0005	0·03	60	5·07	—	—	1·70
(*i*) 12·5	0·004	0·044	11	5·07	5·14	+1·3	—
(*j*) 20	0·0025	0·065	26	5·07	—	—	1·33

DISCUSSION OF RESULTS

By using the absorptiometric technique Ringbom, Siitonen and Skrifvars[1] established the formation of two complexes between quinquivalent vanadium and EDTA—VO_2Y^{3-} (pH 6 to 9) and VO_2HY^{2-} (below pH 3·5). These findings are difficult to understand, since cations are complexed by EDTA and the vanadyl cation is formed in appreciable quantities only beyond the isoelectric point of quinquivalent vanadium,[12,13] *i.e.*, below a pH of about 2. In our investigation, solutions with a low ratio of hydrogen ions to vanadium gave very low vanadyl titres (see curves A in Figs. 1 and 2), which are attributed to incomplete formation of vanadyl ions.

To establish minimum acidity conditions, or rather the minimum ratio of hydrogen ions to vanadium required for the quantitative conversion of vanadate to vanadyl ion in the reaction expressed by equation (1) we studied the hydrolysis of a 0·0017 M aqueous solution of vanadium pentoxide with perchloric acid by means of accurate conductivity measurements (see Fig. 4)—

$$V_2O_5 \text{ aq.} + 2H^+ \rightleftharpoons 2VO_2^+ + H_2O \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

In Fig. 4, curve A, denoting the change in conductivity of the aqueous vanadium pentoxide - perchloric acid system, becomes parallel to curve B—conductivity of perchloric acid—when the ratio of hydrogen ions to vanadium is about 4 to 1, indicating that on further addition of acid no changes occur in the reaction denoted by equation (3). Moreover, at point P, the fall in conductivity from theoretical corresponds approximately to the removal of one proton per vanadium atom.

Although the lower hydrogen ions to vanadium limit is fixed by the minimum acidity requirements for the quantitative conversion of vanadate to vanadyl ions,* the upper limit is governed by the general instability of the metal - EDTA complex in the presence of excess of acid (equation 2), which can be particularly serious with a univalent cation.[14]

In addition, the oxidising power of the vanadyl^V cation increases with increase in hydrogen-ion concentration,[15] thus rendering the risk of a redox reaction between VO_2^+ and EDTA more pronounced.

* This ratio would correspond to 5 to 1 for ammonium metavanadate.

The quantitative character of complex formation between EDTA and the univalent vanadyl^{V} cation near the lower hydrogen ions to vanadium limit was established by a series of accurate conductivity measurements (see Fig. 3) and by volumetric experiments (see Table III) by the indicator method. At the higher acidity limit, complexation is still quantitative when the ratio of hydrogen ions to vanadium is 26 (experiment (*c*), Table III), whereas for a ratio of 36 the experimental end-point is 5 per cent. low, and, for even higher ratios, the colour change is so gradual as to make the end-point undetectable. This is also true when the total acidity is high (experiment (*j*), Table III).

In the indicator method, the concentration of vanadium has an indirect effect. If it is large enough to cause the minimum acidity to drop the pH below 1·8, a satisfactory end-point is not obtained. At the other extreme, for very small amounts of vanadium in solution the metal-indicator colour becomes too faint, even above pH 1·8.

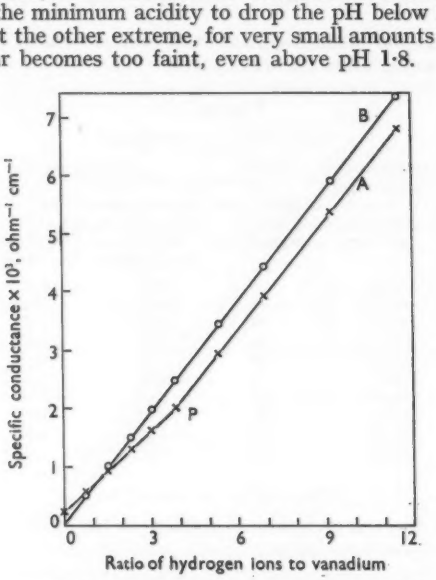

Fig. 4. Conductivity curves at 25° ± 0·001° C for: curve A, solutions of vanadium pentoxide in perchloric acid (0·0017 gram-atom of vanadium per litre); curve B, solutions of perchloric acid

The rather ill-defined breaks in the conductivity curves (Figs. 1 and 2) may be attributed to the low concentrations of the solutions, rendering the conductivity measurements less accurate. This is overcome to a large extent by the improved instrumentation (Fig. 3).

As regards the redox character of the vanadyl^{V} - EDTA system, we found that partial reduction to the blue vanadyl^{IV} ion occurs in solutions of high hydrogen-ion concentration. Around pH 2, this reduction appears to be slow and does not interfere with the titrimetric determination of the vanadyl^{V} ion. Even when the solution is set aside for 7 days, the conductivity break was only 2·2 per cent. high (see Fig. 3).

Although the vanadyl^{V} - EDTA complex seems to be only moderately stable and involves a delicate balance between minimum and maximum acidity requirements, we suggest the method described below for the volumetric determination of vanadium.

Place the vanadium compound, containing between 7·5 and 25 mg of vanadium, in a 250-ml conical flask together with 30 ml of 0·1 M perchloric acid, gently boil the solution, cool, and dilute to about 100 ml. Add 3 drops of indicator solution, and titrate with 0·01 M EDTA until the last trace of orange disappears and the solution is pure yellow.

50·95 g of vanadium ≡ 1000 ml of M EDTA

∴ 100 ml of 0·01 M EDTA ≡ 51 mg of vanadium.

We thank Dr. T. S. West for recommending the use of xylenol orange and for supplying us with samples of xylenol orange and pyrocatechol violet. We also thank the Manchester Oil Refinery for a gift of Puremore 210 light oil.

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Colorimetric Determination of Copper with Zinc *OO*-Di-isopropyl Phosphorodithioate

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Zinc *OO*-di-isopropyl phosphorodithioate is recommended for the colorimetric determination of copper in the range 4 to 40 μg. Beer's law is obeyed, and both the solid reagent and its solution in carbon tetrachloride are stable. The method is rapid and precise, and large excesses of aluminium, antimony, arsenic, barium, cadmium, calcium, cobalt, chromium, ferrous and ferric iron, lead, magnesium, manganese, mercury, nickel, potassium, sodium and zinc do not interfere. Results are high when more than 0·1 mg of bismuth is present and erratic in presence of more than 5 mg of silver.

Of the many reagents recommended for determining copper colorimetrically, cuproine[1] and zinc dibenzyldithiocarbamate[2] are perhaps of most general use. Cuproine is specific, but requires careful control of pH; zinc dibenzyldithiocarbamate (like zinc *OO*-di-isopropyl phosphorodithioate) is non-specific, but applicable over a wide range of acidity. Zinc *OO*-di-isopropyl phosphorodithioate is less subject to interference from antimony, mercury, nickel and silver than is zinc dibenzyldithiocarbamate, but more subject to interference from arsenic and chromium. Bismuth interferes seriously when either of the zinc compounds is used.

Busev and Ivanyutin,[3] in a short paper published in 1957, described the use of potassium *OO*-diethyl phosphorodithioate for the colorimetric determination of copper. We were interested in their paper, inasmuch as zinc *OO*-di-isopropyl phosphorodithioate had been used for several years in this laboratory as a reference substance for determining phosphorus in organic compounds and for the colorimetric determination of dialkyl phosphorodithioates. We therefore decided to investigate its suitability as a colorimetric reagent for copper.

Experimental

Acid solutions containing copper were extracted with a 0·04 or 0·004 per cent. solution of zinc *OO*-di-isopropyl phosphorodithioate in carbon tetrachloride. The extracts obtained were examined with a Spekker absorptiometer, and the results showed that—

(*i*) Beer's law is obeyed over the range 4 to 40 μg of copper when a mercury-vapour lamp and filters transmitting at 4047 A are used. The yellow complex with copper has an absorption maximum at approximately 4200 A.

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(*ii*) Bright sunlight causes the colour to fade slowly; in one experiment, the optical density decreased by 36 per cent. in 3 hours.

(*iii*) A single extraction for 30 seconds removes copper completely.

(*iv*) Wide variation in acidity is permissible. Experiments were carried out in which 50-ml portions of solutions of different acidity and containing 24 μg of copper were extracted with 25-ml portions of a 0·004 per cent. solution of zinc *OO*-di-isopropyl phosphorodithioate. Twenty-two such experiments under the conditions of acidity listed below gave a standard deviation of only $\pm$0·002 unit of optical density.

Acid	Sulphuric	Hydrochloric	Perchloric	Orthophosphoric	Nitric
Acidity of extracted solution	0·1 to 5 N	0·1 to 5 N	0·1 to 5 N	0·1 to 1·5 M	0·1 to 2 N

The colour of the extract from a solution 3 N in nitric acid fades rapidly; for the other acids, no experiments were made at concentrations higher than the maximum values indicated above.

(*v*) Wide variation in the concentration of the zinc *OO*-di-isopropyl phosphorodithioate solution is permissible. In most of the preliminary experiments we used a 0·004 per cent. solution, but studies on interfering ions showed that a 0·04 per cent. solution is better.

(*vi*) Delay between the addition of the zinc *OO*-di-isopropyl phosphorodithioate solution and shaking should be avoided. Experiments were made in which intervals of 0, 2, 5 and 10 minutes elapsed, and the optical-density readings were 0·232, 0·224, 0·216 and 0·191, respectively. The probable explanation for this decrease in optical density is that dialkyldithiophosphoric acid slowly passes into the aqueous layer and there forms the cupric complex. It is known that the latter, in aqueous solution, rapidly changes to a colourless cuprous compound.

(*vii*) The stability of the reagent is excellent. Both 0·004 and 0·04 per cent. solutions of zinc *OO*-di-isopropyl phosphorodithioate were stored for 10 weeks in plain-glass bottles on the laboratory window-sill without deterioration.

Method

Reagents—

Carbon tetrachloride, purified—The use of technical-grade carbon tetrachloride occasionally leads to low results, owing, we suspect, to the presence of traces of phosgene. Purify the solvent by shaking 600 ml of it successively with 100 ml of dilute ammonia solution (1 + 4) and two 100-ml portions of water. Dry the carbon tetrachloride over anhydrous sodium sulphate, and store in a dark-glass bottle.

Zinc OO-*di-isopropyl phosphorodithioate solution, 0·04 per cent. w/v*—Dissolve the necessary amount of solid reagent, prepared as described below, in purified carbon tetrachloride.

Hydrochloric acid, sp.gr. 1·18—Analytical-reagent grade.

Standard copper solution—Dissolve 0·1964 g of hydrated copper sulphate, $CuSO_4.5H_2O$, in water in a 250-ml calibrated flask, acidify with a little hydrochloric acid, and dilute to the mark with water. Dilute 10 ml of this solution, again acidified with hydrochloric acid, to 250 ml; prepare this solution freshly each day, as required.

1 ml $\equiv$ 8 μg of copper.

Preparation of zinc *OO*-di-isopropyl phosphorodithioate—

Weigh 10 g of phosphorus pentasulphide into a Quickfit & Quartz 250-ml conical flask, attach a reflux condenser, add 100 ml of isopropyl alcohol, and heat for 5 minutes or more on a water bath, with frequent agitation, to give a clear solution. Cool to about 40° C, and expel hydrogen sulphide by passing a stream of nitrogen through the liquid for 3 minutes. Add 4 g of analytical-reagent grade zinc oxide, heat on the water bath for 10 minutes, and filter the hot mixture through a sintered-glass funnel (porosity No. 4). Chill the filtrate, separate the crystals that form, and wash them with two small portions of cold isopropyl alcohol. Recrystallise twice from hot isopropyl alcohol, and dry the white crystalline product in air; this procedure yields about 6 g of zinc *OO*-di-isopropyl phosphorodithioate.

Four samples were prepared in this way. Each melted at 145° $\pm$ 2° C and contained proportions of carbon, hydrogen and phosphorus that agreed well with the values calculated from the formula $[(C_3H_7O)_2PSS]_2Zn$. All four samples gave satisfactory results in the colorimetric determination of copper.

PROCEDURE—

Place an aliquot of solution containing 4 to 40 μg of copper in a Pyrex-glass 125-ml separating funnel, and dilute with water to approximately 50 ml, adding sufficient hydrochloric acid, sp.gr. 1·18, to make the diluted solution approximately 4 N in this acid. By pipette, add 25 ml of 0·04 per cent. zinc *OO*-di-isopropyl phosphorodithioate solution to the contents of the separating funnel; fill the pipette by means of suction from a water pump. Without delay, shake the separating funnel vigorously for 30 seconds, and allow the layers to separate. Dry the stem of the separating funnel with a roll of filter-paper, and insert a loose plug of cotton-wool. Measure the optical density of the extract in a 2-cm cell with a Spekker absorptiometer; use a mercury-vapour lamp and a filter transmitting at 4047 A. Alternatively, use any system permitting measurements to be made in the vicinity of 4200 A.

TABLE I

EFFECTS OF VARIOUS CATIONS

The amount of copper taken for determinations by the proposed method was 24 μg; amounts from 10 to 40 μg were used in the zinc dibenzyldithiocarbamate method, as recommended by Johnson.[2] The figures for each cation are the maximum amounts that may be present if the error is not to exceed ± 2 μg of copper

Cation added	Maximum amount permissible in— proposed method, g	zinc dibenzyldithiocarbamate method, g
Al^{3+}	0·5	0·5
Sb^{3+}	0·05*	<0·01
As^{3+}	0·1†	0·5
Ba^{2+}	0·5	—
Bi^{3+}	<0·0001	<0·002
Cd^{2+}	0·5	0·5
Ca^{2+}	0·5	0·5
Co^{2+}	0·01	0·01
Cr^{3+}	—	0·5
Cr^{6+}	0·01	—
Fe^{2+}	0·5	0·5
Fe^{3+}	0·01	<0·5
Pb^{2+}	0·5	0·5
Mg^{2+}	0·5	—
Mn^{2+}	0·5	0·5
Hg^{2+}	0·05	0·0001
Ni^{2+}	0·5	0·05
K^{+}	0·5	—
Ag^{+}	0·005	0·0001
Na^{+}	0·5	—
Zn^{2+}	0·5	0·5

* Colour fades in the presence of antimony, and the optical density must be read with dispatch.
† Solution and reagent shaken for 2 minutes.

CALIBRATION—

Transfer portions of the standard copper solution ranging from 0 to 5 ml to separate 125-ml separating funnels, and add to each portion, from a measuring cylinder, 17·5 ml of hydrochloric acid, sp.gr. 1·18. Dilute each solution with water to approximately 50 ml, add reagent, extract, and measure the colour as described above.

INTERFERENCES

CATIONS—

The effects of twenty cations were studied, and the amounts that may be present when determining 24 μg of copper are listed in Table I, which also shows comparative results for zinc dibenzyldithiocarbamate. Bismuth interferes seriously with the proposed method by forming a complex that absorbs strongly in the near-ultra-violet region; this interference could perhaps be overcome by using a prism or grating spectrophotometer. Silver must not be present in amounts greater than 5 mg.

The effects of the twenty cations were also investigated by means of a modified method, the concentration of hydrochloric acid being decreased from 4 to 0·1 N and that of the zinc *OO*-di-isopropyl phosphorodithioate solution from 0·04 to 0·004 per cent. Several cations interfered to a greater extent when the modified method was used, as shown by the results below.

Cation	Sb^{3+}	Fe^{2+}	Fe^{3+}	Hg^{2+}	Ni^{2+}
Maximum amount permissible for error of ± 2 μg of copper, g	0·0005	0·05	<0·005	0·0001	0·01

Only arsenic interfered less, the permissible amount being 0·5 g.

Interference from bismuth—As much as 0·01 g of bismuth may be present if the acidity of the test solution is kept at 4 N in hydrochloric acid and the concentration of the zinc *OO*-di-isopropyl phosphorodithioate solution is decreased to 0·004 per cent. Interference from ferric iron also disappears under these conditions, and as much as 0·5 g of this ion may be present.

ANIONS—

The results of experiments carried out in presence of nine sodium and potassium salts are summarised in Table II; only molybdate and iodide interfered to a marked extent.

TABLE II

EFFECTS OF VARIOUS SALTS

The figure for each salt is the maximum amount that may be present if the error in determining 24 μg of copper is not to exceed ± 2 μg

Salt added	Maximum amount permissible in proposed method, g
Sodium acetate	0·5
Potassium bromide	0·5
Trisodium citrate	0·5
Potassium iodide	0·01
Sodium molybdate dihydrate	0·05
Sodium nitrite	0·25*
Potassium oxalate monohydrate	0·5
Potassium permanganate	0·5†
Potassium sodium tartrate tetrahydrate	0·5

* Add 0·5 g of potassium permanganate, boil until pale brown in colour, and cool before extraction with reagent solution.

† Boil until pale brown in colour, and cool before extraction.

CONCLUSION

In developing this method, we had in mind its eventual application to calcium phosphates and decided on that account to use hydrochloric acid solutions. It seems probable from the information under "Experimental" that other acids could equally well be used in determining copper colorimetrically.

We thank Mr. S. F. Holder for suggesting this investigation and for his interest in its progress and the Directors of Albright & Wilson (Mfg) Ltd. for permission to publish this paper.

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Notes

THE COMBUSTION OF ORGANIC COMPOUNDS BY IGNITION IN OXYGEN: THE DETERMINATION OF CARBON AND HYDROGEN

THIS Note is a preliminary report of an investigation in which the flask combustion technique was used to determine carbon and hydrogen in organic substances.

The first serious attempt to determine carbon and hydrogen involved igniting a mixture of the weighed sample with a known weight of potassium chlorate. This ignition process, developed by Gay-Lussac and Thenard in 1810, was carried out by dropping the pelleted mixture into a closed tube held in the vertical position and heated at its lower end by the flame of a spirit lamp. When the pellets came into contact with the hot end of the tube, ignition occurred, with the production of oxygen, carbon dioxide and water vapour. These products were collected over mercury, and the amounts of carbon and hydrogen in the sample were determined by gasometric analysis of the mixture.

The obvious drawback to this method was the crude way in which the weights of products were obtained, and it was by no means certain that the carbon dioxide had been quantitatively collected. Because of this, the principle of combustion now in common use was evolved by Liebig from earlier work by Berzelius and has not changed, even with its application by Pregl to the combustion of a few milligrams of material. Over the years, the simple operation of direct ignition has been lost, and the method has become one of a rather complex nature, in which complicated and expensive apparatus is used to burn the few milligrams of substance involved. On the micro scale, great effort has been, and still is being, directed towards hastening the method of analysis and making the combustion operation less tedious; rapid combustion,[1] the use of catalysts, such as thermally decomposed silver permanganate,[2] and automation of the combustion procedure are typical examples of the means used to achieve these aims.

At the turn of the century, some workers attempted to establish a method in which the sample was ignited in a Berthelot bomb under pressure of oxygen (20 atmospheres) by means of an electrically heated platinum wire. After combustion, the mixture of carbon dioxide and water vapour was swept out with a current of purified air and analysed. This method did not become established because it had some faults; for example, the presence of nitrogen oxides and oxidation products of the halogens and sulphur caused complications. In view of the success of Hempel's oxygen flask combustion technique, as adapted to the micro scale by Schöniger[3] and now an established procedure, an attempt has been made to adapt this technique to the determination of carbon and hydrogen.

Some preliminary experiments have established a simple and rapid method of determining carbon and hydrogen on the micro scale, and this method has overcome the need for using a baffle-chamber combustion tube.[1]

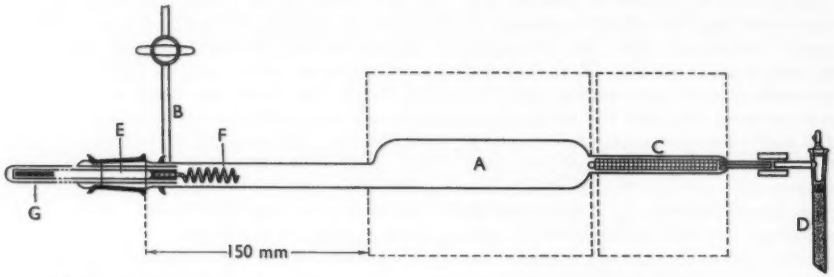
In the first instance, the weighed sample (about 3 mg) was ignited by passing an electric current through a platinum-wire coil placed round the sample boat held in a spherical 200-ml flask filled with oxygen. After ignition, the carbon dioxide and water were swept out by passing a stream of purified oxygen at the rate of 30 to 35 ml per minute and determined gravimetrically. The results were not satisfactory, being low and inconsistent; the losses were not caused by leakage from the flask, but by incomplete combustion.

Malissa[4] recently described a method in which the sample was decomposed in a fast flow of oxygen at a high temperature. The carbon dioxide formed was absorbed in sodium hydroxide solution and determined by means of a conductivity measurement. In the light of this work, further experiments were carried out in which the sample was introduced directly into a combustion chamber heated by a furnace (at about 750° C), oxygen being allowed to flow through the chamber at 35 ml per minute. After continued sweeping for 15 minutes, the carbon dioxide and water collected in weighed absorption tubes were determined. In these experiments, the results for hydrogen were satisfactory, but those for carbon were low by about 2 per cent. Finally, it was established that correct results for carbon and hydrogen could be obtained with the required accuracy by introducing the sample into the oxygen-filled chamber, but without maintaining the flow of oxygen during combustion. As soon as the sample had burned, a matter of a few seconds after its introduction into the hot zone, oxygen was passed into the chamber to sweep out the water and carbon dioxide, which were absorbed in the conventional manner for subsequent gravimetric determination.

Description of combustion apparatus

The combustion tube had the shape shown in Fig. 1 and was constructed from quartz tubing having a wall-thickness of 1·8 to 2 mm. The chamber, A, was 150 mm long and about 33 mm in internal diameter; it had a volume of about 130 ml at room temperature and contained about 50 ml of oxygen at its operating temperature. The length of tubing from the chamber to the ground joint was 150 mm, and its bore was about 14 mm. Oxygen was admitted via a side-arm, B, 150 mm in length, sealed to the tube just below the ground joint. The exit from the chamber consisted of a 90-mm length of tubing, C (8 to 9 mm bore), provided with a "beak" 30 mm long and 3·5 mm in diameter. A roll of silver gauze was placed in the exit tube to retain halogen and sulphur oxidation products. The chamber was heated by an electric furnace at 720° to 750° C, and the temperature of the silver gauze was maintained at about 550° C by another electric furnace.

Water and carbon dioxide were absorbed in Flaschenträger tubes packed with suitable reagents; when nitrogen-containing compounds were analysed, the oxides of nitrogen were retained by manganese dioxide in an absorber[1] placed between the two Flaschenträger tubes.



A = Combustion chamber
B = Side-arm
C = Exit tube containing silver gauze
D = Flaschenträger tube for absorbing water
E = Sample holder
F = Platinum-wire coil
G = Borosilicate-glass sleeve

Fig. 1. Diagram of combustion apparatus

The oxygen was purified by passing it through a pre-heater and a large U-tube containing soda asbestos and anhydrone and was passed into the combustion tube via a stopcock attached with rubber tubing to side-arm B. The flow of gas was controlled at 50 ml per minute by means of a precision screw-clip and a pressure regulator. The flow was measured with a Rotameter reading to 200 ml of oxygen per minute.

The sample was introduced into the combustion chamber by means of a special holder, E. This consisted of a quartz tube, 330 mm long and 6 mm external diameter, having a coil of platinum wire, F, sealed into one end. The coil was made from a piece of 16 s.w.g. wire about 170 mm in length formed into a spiral (about 25 mm long) by winding it round a piece of glass rod 8 to 8·5 mm in diameter. The sample boat was placed in the coil for introduction into the chamber. The other end of the quartz tube was provided with an iron core of length 70 mm kept in position by an indentation in the wall of the tube. The holder was placed inside an elongated borosilicate-glass sleeve, G, sealed into the ground-joint cap closing the mouth of the combustion tube. The sleeve projected 20 mm beyond the edge of the joint, as shown in Fig. 1. Tube E fitted into sleeve G in a way such that it would slide in and out easily, without friction. The sample was moved into the centre of the combustion chamber in one operation by application of a magnet to the iron core. In preliminary experiments, the holder was pushed into the chamber by hand, the tube being fixed in a rubber sleeve attached to the ground cap. It was found, however, that high blank values were obtained after the apparatus had been used because of a leak through the rubber sleeve.

Method

The absorption tubes were conditioned as for a rapid combustion[1] for 10 minutes, with oxygen flowing at 50 ml per minute. Blank determinations were carried out, and it was established that the blank value for water was 0·05 mg; the blank value for carbon dioxide was negative.

COMBUSTION—

The weighed absorption tubes were attached to the combustion tube, and the ground cap was carefully removed from the mouth of the tube. Oxygen was allowed to escape via the open tube, and then the sample boat was placed in the platinum spiral of the holder, which was still completely in the sleeve. The holder was replaced, and the joint was secured with springs. Oxygen was passed through the apparatus at 50 ml per minute for 1 minute, the stopcock was turned off, and, after a few seconds, the inlet tap - stopper of the water-absorption tube was closed.

With the aid of a magnet, the sample holder was then pushed, in one movement, into the centre of the combustion chamber. As soon as the sample had burned, the stopcock connected to the side-arm was opened to permit oxygen to enter the combustion tube, the inlet tap - stopper of the water-absorption tube was opened, and the apparatus was swept out with oxygen for 13 minutes at the rate of 50 ml per minute. The absorption tubes were then disconnected as described for the rapid-combustion method.[1]

TABLE I

RESULTS FOR CARBON AND HYDROGEN BY THE PROPOSED METHOD

Compound	Weight of sample, mg	Carbon content found, %	Hydrogen content found, %	Calculated carbon content, %	Calculated hydrogen content, %
Anthracene	2·127	94·62	5·78	94·36	5·66
	2·200	94·46	5·59		
	3·745	94·25	5·54		
Naphthalene	3·545	93·62	6·30	93·75	6·25
	4·283	93·84	6·22		
Benzene	3·675	92·35	7·86	92·26	7·74
	3·900	92·18	7·92		
Octan-2-ol	2·481	73·70	13·75	73·78	13·93
	3·154	73·97	13·97		
Benzoic acid	3·227	68·75	5·06	68·90	4·95
	3·230	69·02	4·95		
Succinic acid	3·896	40·87	5·09	40·68	5·08
	4·678	40·50	5·03		
Sucrose	3·748	42·01	6·38	42·10	6·48
	3·886	42·20	6·36		
Phenacetin	3·313	67·09	7·29	67·04	7·26
	3·498	67·21	7·37		
p-Nitroaniline	3·061	52·24	4·36	52·13	4·38
	3·493	52·26	4·59		
p-Chlorobenzoic acid	2·684	53·76	3·32	53·68	3·22
	3·573	53·75	3·28		
Sulphonal	3·642	36·92	7·13	36·81	7·06
	4·685	36·74	7·10		
Triphenylphosphine	3·184	82·60	5·82	82·41	5·76
	3·847	82·52	5·70		

DISCUSSION OF THE METHOD

The method has been tested with several types of organic compounds, and typical results are shown in Table I. Samples containing carbon, hydrogen, oxygen and nitrogen (the last-named present as NH_2, NO or NO_2) have been analysed satisfactorily by using a combustion tube without the exit tube containing silver gauze attached; the chamber was heated with the flame of a large bunsen burner, giving an operating temperature of 725° to 750° C. In these experiments, the chamber was enclosed in a sheath of nickel foil, the ends of which were encased in plates of thick asbestos sheet.

No difficulty was experienced in analysing liquids, *e.g.*, benzene. Liquid samples were weighed in the usual form of capillary tube and placed in a short piece of quartz tubing 65 mm long and 5·5 to 6 mm in bore. The quartz sleeve was placed in the platinum spiral for introduction into the combustion chamber.

Triphenylphosphine has been analysed satisfactorily by covering the sample in the boat with tungstic oxide,[5] and the analysis of organo-metallic compounds is being investigated. Promising results have been obtained with fluorine-containing substances, *e.g.*, *m*-trifluoromethylbenzoic acid; such samples were covered with magnesium oxide.[6] When a flux or reagent was used, the sample was weighed in a long narrow boat, which was then placed in a quartz sleeve before insertion into the platinum spiral. Initially, tests were carried out in which the sample was introduced into the hot zone by means of a short form of the holder E, which was placed immediately behind the cap of the tube, so eliminating sleeve G. However, this simple method of moving the sample holder into the hot zone was not always satisfactory, as the holder tended to rotate when moved forward by means of the magnet, and sample or added reagent was tipped out into the combustion tube.

It is hoped that this simple combustion procedure will eventually be able to cope with all types of compounds difficult to analyse by other established methods, and work is proceeding with this aim in mind. The method of combustion provides a means of analysing the "difficult" organo-metallic compounds now being prepared, because suitable reagents can be sought to assist decomposition; the use of such reagents, coupled with a high temperature of combustion, permits quantitative oxidation. The possibility of increasing the rate of flow of oxygen to 100 ml or more per minute is being considered, as such an increase would cut the sweeping-out period by half. Other aspects being investigated are the determination of the water and carbon dioxide manometrically, so that the method could be extended to the determination of compounds containing carbon-14. The method of combustion also provides a simple way of decomposing samples for the determination of the halogens and sulphur, particularly when combustion is found to be difficult by the oxygen flask procedure. Work on these methods is in progress, and it is hoped that a full account of the investigations will be published later.

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THE DETERMINATION OF NITRITE IN WATER

Most methods described for the determination of nitrite depend on diazotisation. The diazonium compound formed is usually determined colorimetrically, either by direct measurement[1] or after being coupled with a suitable reagent. The latter principle is employed in the Griess - Ilosvay method, various modifications[2] of which are commonly used in water analysis. This method, although reliable, suffers from a number of disadvantages: the colour formed is rather unstable, the reagent, 1-naphthylamine, deteriorates when stored, and samples may require excessive dilution on account of the small range over which results are reproducible. The method described in this Note is largely free from these disadvantages.

Garner, Baumstark, Muhrer and Pfander determined nitrate by a method,[3] later improved by Hill, Pivnick, Engelhard and Bogard,[4] in which a bacterial culture was used to reduce the nitrate to nitrite; this ion was then determined as in the Griess - Ilosvay method, except that *N*-(1-naphthyl)ethylenediamine was used instead of 1-naphthylamine. Sulphanilic acid and *N*-(1-naphthyl)ethylenediamine had previously been used for the indirect determination of nitrogen dioxide by Saltzman,[5] who found that these reagents were more satisfactory than the Griess - Ilosvay reagents or the combination of sulphanilamide and *N*-(1-naphthyl)ethylenediamine introduced by Shinn.[6]

By modification of the conditions used by Garner and his co-workers, we have developed a convenient method for determining nitrite in water. The reagents are added separately, instead of together as in the earlier procedures, and the acidity of the solution is established with potassium

hydrogen sulphate, which has a greater buffering capacity than the acetic acid normally used. Beer's law is obeyed up to 2 μg of nitrite-N, and amounts up to 8 μg can be determined with use of a calibration graph. (The official Griess - Ilosvay procedure[2] formerly used in this laboratory does not give good reproducibility for amounts of nitrite-N greater than 1 μg.) The colour produced is more intense by 13 per cent., and blank values are less than half of those obtained in the official procedure. The *N*-(1-naphthyl)ethylenediamine hydrochloride solution can be stored in the dark for at least 2 months.

TABLE I

COMPARISON OF RESULTS BY PROPOSED AND OFFICIAL METHODS

Sample	Nitrite added (as N), p.p.m.	Total nitrite (as N) found by— proposed method, p.p.m.	Total nitrite (as N) found by— official method, p.p.m.	Bias (as N) of— proposed method, p.p.m.	Bias (as N) of— official method, p.p.m.
Percolating-filter effluent A	0·0	3·0	3·0	—	—
	4·0	7·0	7·0	0·0	0·0
	6·0	9·1	—	+0·1	—
	8·0	—	11·1	—	+0·1
Percolating-filter effluent B	0·0	0·13	0·14	—	—
	0·1	0·23	0·23	0·0	−0·01
	0·2	0·33	0·33	0·0	−0·01
	0·3	0·43	0·42	0·0	−0·02
Thames water from Tilbury (salinity 13·4 parts per thousand)	0·0	0·15	0·17	—	—
	0·2	0·36	0·37	+0·01	0·0
	0·4	0·56	0·57	+0·01	0·0
	0·6	0·77	0·76	+0·02	−0·01
	0·0	0·15*	—	—	—
	0·2	0·34*	—	−0·01	—
	0·4	0·53*	—	−0·02	—
	0·6	0·72*	—	−0·03	—
Percolating-filter effluent C	0·0	0·042	0·040	—	—
	0·1	0·142	0·135	0·0	−0·005
	0·15	0·194	—	+0·002	—
	0·2	0·241	0·234	−0·001	−0·006
	0·3	—	0·339	—	−0·001
	0·0	0·042*	—	—	—
	0·1	0·140*	—	−0·002	—
	0·15	0·183*	—	−0·009	—
Digester effluent containing phenols	0·0	0·79	0·82	—	—
	0·4	1·21	1·24	+0·02	+0·02
	0·8	1·58	1·61	−0·01	−0·01
	1·2	2·00	2·02	+0·01	0·0
	0·0	0·73*	—	—	—
	0·4	1·11*	—	−0·02	—
	0·8	1·48*	—	−0·05	—
	1·2	1·88*	—	−0·05	—
Sea water from Worthing	0·0	0·008	0·004	—	—
	0·05	0·055	0·051	−0·003	−0·003
	0·1	0·108	0·101	0·0	−0·003
	0·15	0·157	0·144	−0·001	−0·010
	0·0	0·008*	—	—	—
	0·05	0·060*	—	+0·002	—
	0·1	0·112*	—	+0·004	—
	0·15	0·162*	—	+0·004	—

* Large sample taken; optical density measured in 1-cm cells.

No quantitative statements on the stability of the colour produced in the Griess - Ilosvay method have been found in the literature, although there are reports of precipitation or intensification of the colour. In a 24-hour test of the stability of the colour produced by the official procedure[2] in this laboratory, the colour derived from a standard solution of pure nitrite decreased by 2 per cent. and those from sewage effluents showed increases ranging up to 6 per cent. Under similar conditions, the colours obtained in the proposed method remained within 2·5 per cent. of their initial intensities for 2 days.

When typical samples were analysed by both methods, there was good agreement between the two sets of results, some of which are shown in Table I. The samples, which included sea

water and water from the Thames Estuary, were then fortified with known amounts of standard nitrite solution and again analysed. The increments observed normally agreed with the calculated values, but certain samples initially extremely low in nitrite (less than 0·1 p.p.m. of nitrite-N) showed a bias towards low recovery by both procedures. For samples from the Stevenage Brook, this effect is associated with warm and dry weather conditions. A similar bias was observed with effluents from the biological treatment of farm wastes containing phenolic disinfectants; for these effluents, however, the bias could be removed by taking much smaller samples for the determination.

Although most of the optical-density measurements were made at 550 mμ with a spectrophotometer, an identical calibration graph was obtained when a Spekker absorptiometer and a Spectrum Green No. 604 filter were used.

Method

Reagents—

Sulphanilic acid solution—Dissolve 27·2 g of potassium hydrogen sulphate and 3·46 g of sulphanilic acid in water, and dilute to 1 litre.

N-(1-*Naphthyl*)*ethylenediamine hydrochloride solution, 0·04 per cent.*—Store in the dark.

Procedure—

Clarify the sample, if necessary, by filtration or centrifugation, measure a suitable volume (not more than 30 ml) into a 50-ml calibrated flask, and adjust the temperature to between 20° and 30° C. (For effluents containing phenols, use the smallest practicable sample; otherwise, take a sample containing between 0·2 and 1·0 μg of nitrite-N unless the concentration is high, when up to 8 μg may be tolerated.) Dilute to at least 10 ml, add 2·5 ml of sulphanilic acid solution, and set aside for 10 minutes. Add 2·5 ml of *N*-(1-naphthyl)ethylenediamine hydrochloride solution, set aside for 20 minutes, dilute to the mark, and measure the optical density at 550 mμ against a reagent blank solution; use 4-cm cells unless the amount of nitrite-N present exceeds 1·5 μg, when 1-cm cells are satisfactory. For the most accurate work, or with turbid or highly coloured samples, prepare an additional blank solution by carrying out the procedure described above, but omit the *N*-(1-naphthyl)ethylenediamine hydrochloride solution. Measure the optical density against distilled water, and subtract the value from the optical density found in the test. This correction is usually negligible.

Ascertain the nitrite content of the sample by reference to a calibration graph plotted from the results obtained for standard solutions of nitrite; we have found that solutions of analytical-reagent grade sodium nitrite (previously dried at 110° C) in freshly distilled water are suitable for this purpose and are stable for at least 1 month. The purity of the sodium nitrite was checked by titration against a standard solution of potassium permanganate.

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INTERFERENCE BY CHLORIDE ION IN THE DETERMINATION OF TRYPTOPHAN BY SHAW AND McFARLANE'S METHOD

Shaw and McFarlane developed a convenient colorimetric method for determining tryptophan in proteins.[1] The method is an adaptation of the Hopkins - Cole reaction[2] and has in general proved satisfactory in this laboratory, where it has been extensively used for analysing proteins and in studies on the preservation of tryptophan by sulphur dioxide during acid hydrolysis of proteins.[3]

In work on the rates of hydrolysis of whole casein and the various fractions of the casein complex, by proteolytic enzymes and by acids, 4 g of Warner casein (containing 14·1 per cent. of nitrogen[4]) and 80 ml of 3 N hydrochloric acid were boiled under reflux. Samples (3 ml) of the mixture (casein hydrolysate No. 1) were taken at various time intervals and were analysed for tryptophan by Shaw and McFarlane's method[1] and for total nitrogen by a micro Kjeldahl method.[5] A sample of the casein used was also analysed and found to contain 0·10 g of tryptophan per g of nitrogen. This experiment was then repeated with 3 N sulphuric acid instead of hydrochloric acid; the results were—

Time of heating, hours ..	0·25	0·5	0·75	1·0	1·25	1·5	2·0	2·25	2·50	2·75	3·0
Tryptophan found in HCl hydrolysate, g per g of nitrogen..	0·128	0·124	0·122	0·118	0·110	0·112	0·106	0·099	0·094	0·090	0·084
Tryptophan found in H_2SO_4 hydrolysate, g per g of nitrogen ..	0·099	0·098	—	0·097	—	0·092	0·084	—	0·074	—	0·066

These figures show that the tryptophan content of the hydrochloric acid hydrolysate was (*a*) higher than that of the sulphuric acid hydrolysate and (*b*) did not attain that of the unhydrolysed casein (0·10 g of tryptophan per g of nitrogen) until the casein - acid mixture had been heated for more than 1·5 hours. This indicated that the hydrochloric acid had interfered with the determination of tryptophan.

Accordingly, a mixture of 1·0 g of casein and 20 ml of a 20 per cent. solution of sodium hydroxide was heated for 20 minutes on a steam-bath. (This is the first step in the determination of tryptophan in proteins by Shaw and McFarlane's method.) The volume of the resulting solution was adjusted to 25 ml, and various amounts of sodium chloride were added to 1-ml portions of the solution, each containing 0·04 g of casein. Tryptophan was determined in these samples by Shaw and McFarlane's method; the results were—

NaCl added, millimoles	0·0	0·1	0·3	0·5	1·0	2·0	4·0	6·0	8·0
Tryptophan found, g per g of nitrogen ..	0·100	0·102	0·118	0·137	0·131	0·132	0·131	0·128	0·124

which suggested that the presence of chloride had increased the intensity of the Hopkins - Cole reaction.

Lesuk[6] has reported that chloride ions increase the intensity of the colour produced by the reaction of tryptophan with *p*-dimethylaminobenzaldehyde.[7] Spies[8] was able to overcome interference from chloride ions by treating the tryptophan-containing solutions with silver sulphate. Attempts were therefore made to remove chloride ion by adding silver sulphate to solutions prepared by treating casein with a 2·0 per cent. solution of sodium hydroxide according to Shaw and McFarlane's procedure. However, removal of chloride ion in this manner was unsatisfactory in so far as it led to the precipitation of protein.

A sample of casein hydrolysate No. 1 prepared in the previous experiments was extracted with di-2-ethylhexylamine solution (1 part by volume of di-2-ethylhexylamine *plus* 10 parts by volume of chloroform); this removed virtually all the chloride. The hydrolysate was extracted with freshly distilled chloroform to remove the di-2-ethylhexylamine and was then heated on a steam-bath to remove chloroform. The hydrolysate, which then contained no detectable chloride ion or chloroform, was analysed for tryptophan by Shaw and McFarlane's method. It was observed that the blanks (extracted hydrolysate to which no glyoxylic acid had been added) were faintly pink in colour, so that it was impossible to obtain an accurate value for the tryptophan content of the hydrolysate. The pink colour appearing in the blanks had the same peak absorption as did that produced by the action of glyoxylic acid on tryptophan.

These observations show that Shaw and McFarlane's method is subject to interference from chloride ion and that this possible interference should be taken into account when the method is used.

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DETERMINATION OF COPPER IN PLATINUM JEWELLERY ALLOYS

HAYWOOD and Sutcliffe's method[1] for determining copper in steel with use of biscyclohexanone oxalyldihydrazone has been extended, with certain essential modifications, to the determination of 0·05 to 6 per cent. of copper in platinum jewellery alloys. As only a small amount of sample is required, the method is particularly suitable for application to delicate articles of jewellery, for which binary alloys of platinum and copper are commonly used. The proposed direct procedure, based on spectrophotometric measurement of the blue copper - reagent complex, is simple in comparison with older methods.

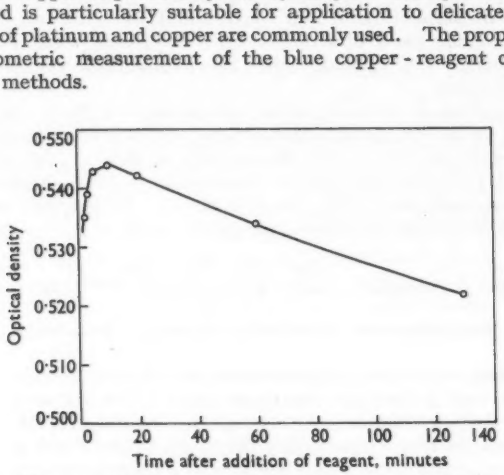

Fig. 1. Variation of optical density of the copper - biscyclohexanone oxalyldihydrazone complex with time. (The values are for a copper - platinum alloy containing approximately 5·5 per cent. of copper)

EXPERIMENTAL

In preliminary experiments, it was found that Haywood and Sutcliffe's method required certain modifications for our purpose. It was established that—

(*i*) The addition of ammonia solution must be deferred until the final-aliquot stage to avoid precipitation of ammonium chloroplatinate.
(*ii*) Accurate adjustment of pH is necessary. This can be achieved by using neutral red indicator as recommended by Wetlesen and Gran.[2,3]
(*iii*) The biscyclohexanone oxalyldihydrazone solution must be freshly prepared and added after the final adjustment of pH.
(*iv*) The blue colour of the complex attains a maximum in about 7 minutes, after which there is slight progressive fading (see Fig. 1). Results are satisfactory, however, provided that the optical density is measured after a fixed interval from the time that the reagent solution is added; the recommended interval is 20 minutes.

The relationship between copper content and optical density found by the proposed procedure is linear; an optical density of 1·000 (4-cm cell) is equivalent to 105 μg of copper in the final solution.

Method

Reagents—

Hydrochloric acid, sp.gr. 1·16.

Nitric acid, sp.gr. 1·42.

Citric acid solution—Dissolve 10 g of citric acid in 200 ml of water.

Ammonia solution—Dilute 100 ml of ammonia solution, sp.gr. 0·880, to 400 ml with water.

Neutral red indicator solution—Dissolve 0·05 g of neutral red in 100 ml of water.

Copper reagent solution—Dissolve 0·1 g of biscyclohexanone oxalyldihydrazone in a mixture of 10 ml each of industrial methylated spirit and hot water, dilute to 200 ml, and filter if necessary.

Procedure—

Weigh 0·02 g of sample in the form of fine powder or drillings into a 150-ml beaker, add 7·5 ml of hydrochloric acid and 2·5 ml of nitric acid, cover the beaker, and heat on a hot-plate until solution is complete; add further portions of hydrochloric and nitric acids if necessary. (Platinum - iridium alloys may require modified treatment to effect solution.) When the sample has dissolved, remove the cover-glass, evaporate almost to dryness, add about 0·05 g of sodium chloride and a few drops of hydrochloric acid, and evaporate to dryness. Dissolve the residue in a little water, add 5 ml of hydrochloric acid, and dilute to 100 ml in a calibrated flask. Transfer a suitable aliquot of this solution (5 ml for samples containing 2 to 6 per cent. of copper) to a second 100-ml calibrated flask, add 5 ml of citric acid solution and 2 drops of neutral red indicator solution, and dilute to about 70 ml. Add ammonia solution until the indicator just turns yellow, and then add 1 drop in excess. Add 20 ml of freshly prepared copper reagent solution, with thorough mixing, dilute to the mark, set aside for exactly 20 minutes, and then measure the optical density at 5950 A in 4-cm cells with a Uvispek spectrophotometer or similar instrument. Carry out a reagent blank determination concurrently with the sample determination, and apply any necessary correction. Determine the copper content of the sample from the corrected optical density by reference to a graph previously prepared from measurements made on solutions of known composition. (The solutions used in preparing the graph should have approximately the same content of platinum as encountered in the determination.)

Table I

Copper contents found in standard alloys

Alloy No.	Nominal copper content, %	Aliquot of alloy solution taken, ml	Copper content found, %
1	3·5	5	3·47
2	3·0	5	2·96
3	2·5	5	2·55
4	1·0	5	1·00
5	0·5	5	0·50
		15	0·51
6	0·2	15	0·23
		25	0·21
7	0·1	25	0·11
8	0·05	25	0·057

Results

The reproducibility of results by the proposed method is good. A series of single determinations carried out on a copper - platinum alloy on successive days gave results of 5·55, 5·53, 5·53, 5·52 and 5·55 per cent. of copper.

Haywood and Sutcliffe found that only iron, nickel, chromium and cobalt interfered with their method.[1] With platinum jewellery alloys, iron and nickel would have negligible effect on the results, whereas chromium and cobalt are unlikely to be present in amounts sufficient to cause any significant interference. The effects of some other elements occasionally found in small amounts were, however, studied; none was found to interfere. To each of five samples of a copper - platinum alloy containing approximately 5·5 per cent. of copper was added a solution of the

element to be tested, the amount of element added being equivalent to a concentration of 5 per cent. in the alloy; the results were—

Element added	None	Gold	Palladium	Rhodium	Ruthenium	Iridium
Copper content found, % ..	5·55	5·53	5·55	5·53	5·57	5·54

The results obtained when the proposed procedure was applied to eight standard copper - platinum alloys are shown in Table I.

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THE PHOTOMETRIC DETERMINATION OF SILICA IN ROCKS AND REFRACTORY MATERIALS

Investigations by Strickland[1] and Ringbom, Ahlers and Siitonen[2] have demonstrated the utility of the yellow α-form of molybdosilicic acid for the photometric determination of silica in rocks and refractory materials. At low acidity, the α-form is stable; the β-form is developed at high acidity and slowly changes to the α-form. Both forms yield blue solutions when reduced by 1-amino-2-naphthol-4-sulphonic acid[3] or *p*-methylaminophenol sulphate,[4,5] and optical-density measurements are made at about 810 mμ. In general, the sensitivity of this method is much too high for rocks containing 35 to 95 per cent. of silica. The great dilution involved may be a source of error, and the strongly acid solution results in initial development of the β-form of the acid. Further, the time elapsing between development of the yellow molybdosilicic acid and its reduction is critical, and errors may also arise from deterioration of the reducing solution and the addition of reagents to mask iron.

In the procedure described below, the sample is fused with sodium hydroxide,[3,5] and the cooled melt, instead of being dissolved in a solution of disodium ethylenediaminetetra-acetate,[2] is taken up in dilute sulphuric acid. This leads to much clearer solutions, particularly for rocks rich in iron. The pH of a suitable portion of the solution is then adjusted to between 3·0 and 3·1, as previously recommended,[2] before the yellow molybdosilicic acid is developed. The effect of iron is overcome by measuring the optical density of each sample solution against a blank prepared from that sample rather than against distilled water or a "fusion" blank. A correction is made for P_2O_5, which is seldom present to an extent greater than 0·5 per cent. in most rocks.

Method

Spectrophotometry—

All optical-density measurements were made in matched 10-mm glass cells with a Unicam SP500 spectrophotometer. In accordance with the method described by Ringbom and Österholm,[6] the concentrations of the final solutions were adjusted so that the optical densities were between 1·0 and 1·6 at a sensitivity of 1 to 10.

Glassware—

Calibrated flasks should be set aside overnight filled with diluted sulphuric acid (1 + 1) and thoroughly rinsed with water just before use. They should never be allowed to become dry and should be stored filled with water.

Reagents—

All reagents should be stored in polythene bottles.
Sodium hydroxide pellets.
Sulphuric acid, 2·5 N.

Buffer solution—Dissolve 200 g of chloroacetic acid in water, and dilute to 1 litre. Slowly add diluted ammonia solution (1 + 1), with stirring, until the pH of the cold solution is between 3·15 and 3·20 (approximately 160 ml of the ammonia solution will be needed).

Ammonium molybdate solution—Prepare a solution containing 35·3 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ per litre.

PROCEDURE—

Place 150-mg portions of standard and samples (weighed to the nearest 0·01 mg) in separate 30-ml silver crucibles, add 1·5 g of sodium hydroxide pellets to the contents of each crucible, and heat the covered crucibles in a muffle furnace for 8 to 10 minutes at 800° C. When cool, half fill each crucible with water, and warm gently on the top of a steam-bath until most of the cake has broken up. Transfer the contents of each crucible to a 500-ml calibrated flask containing about 250 ml of water and 20 ml of 2·5 N sulphuric acid, scrub the crucible and lid with water and then with 5 ml of 2·5 N sulphuric acid to remove any adhering material, and add the rinsings to the contents of the flask. Dilute the solution to the mark with water, and shake thoroughly. Transfer the solution to a dry polythene bottle, insert a stopper, and set aside to clear for 3 or 4 hours or overnight.

TABLE I

SILICA CONTENTS FOUND BY PROPOSED METHOD

For N.B.S. samples certified values are shown in columns 2, 3 and 4; for rocks G-1 and W-1 the values in these columns are those recommended by various workers[7,8,9]

Sample	Total iron present, as Fe_2O_3, %	P_2O_5 present, %	SiO_2 present, %	SiO_2 found by proposed method, %
Burnt refractory (N.B.S. No. 76) ..	2·38	0·069	54·68	54·5(1) 54·6(5) 54·6(0) 54·5(8)
Plastic clay (N.B.S. No. 98)	2·05	0·08	59·11	59·1(9) 59·0(7) 59·0(5)
Granite, G-1	1·96	0·09	72·65	72·4(7) 72·5(1) 72·5(8) 72·5(5)
Diabase, W-1	11·10	0·126	52·64	52·5(1) 52·5(9) 52·5(7) 52·6(1)

By pipette, place 25-ml portions of standard and sample solutions in separate 150-ml beakers, add 15 ml each of buffer and ammonium molybdate solutions, and mix thoroughly. Heat the beakers in a bath of boiling water for 20 minutes, cool to room temperature, transfer each solution to a 100-ml calibrated flask, and dilute to the mark with rinsings from the beaker. Set these solutions, which must all have the same pH (3·0 to 3·1), aside for 12 hours or overnight. Prepare blank solutions for the standard and each sample in exactly the same way, but omit the addition of ammonium molybdate solution and subsequent heating. Measure the optical density of each solution against the appropriate blank solution.

RESULTS

The standard used throughout was National Bureau of Standards sample No. 99 (soda feldspar), which contains 68·66 per cent. of SiO_2, 0·067 per cent. of Fe_2O_3 and 0·142 per cent. of P_2O_5; this standard gave an optical density of 1·498. The lowest working concentration was about 45 per cent. of silica (optical density of 1·0) and the upper limit about 75 per cent. (optical density of 1·6). This range of concentrations covers a large proportion of common igneous rocks, and suitable adjustments to the final concentration can be calculated for samples having silica contents outside this range.

The effect of phosphorus pentoxide was determined by adding this constituent to the standard up to a concentration of 3 per cent. It was found that the presence of 1 per cent. of phosphorus

pentoxide increased the optical density by 0·015 (equivalent to 0·75 per cent. of silica), and the results for silica in the samples were corrected accordingly.

The results of several separate determinations of silica in two National Bureau of Standards refractory samples and two thoroughly investigated rocks, G-1 and W-1, are shown in Table I.

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Book Reviews

Mass Spectrometry and its Applications to Organic Chemistry. By J. H. Beynon. Pp. xii + 640. Amsterdam, London, New York and Princeton: Elsevier Publishing Co.; London: D. Van Nostrand Co. Ltd. 1960. Price 120s.

Various combinations of atoms of the elements can be found to give, as is well known, individual molecules having the same nominal mass number, to the nearest whole number. In fact, since the nuclear packing fraction is different for each isotopic species, the exact mass numbers of these molecules differ to a small extent from each other according to the particular atomic combination making up the molecule. The author of this new book on mass spectrometry observed some years ago that, if the mass spectrometer could be used to distinguish such small differences in mass, it should not be difficult to devise semi-empirical rules for determining the molecular formula of an unknown organic compound. Having obtained the molecular formula, one could then proceed further to an elucidation of the structural formula by a study of the type and abundance of the fragment ions observed in the mass spectrum of the compound. With more complicated molecules, one might have also to consider the information supplied by formation of metastable ions, the possible existence of doubly-charged ions, appearance-potential studies and so on to describe the structure. Nevertheless, it would appear that the mass spectrometer could be applied on a wider basis to chemical analysis, for qualitative as well as quantitative determination.

The author's observation was opportunely made, at a time when the mass spectrometer had already established itself as a powerful analytical instrument in hydrocarbon chemistry and when considerable pressure was being exerted by the users of these instruments on those concerned with instrument design to extend considerably the available mass resolution. In these instances, all the compounds present in the samples could be identified in other ways. The desire was for facility to undertake quantitative analysis in a region of higher molecular weight.

In the development of this field of application—qualitative analysis—the author has made considerable contributions. It is to be expected, then, that in his book on mass spectrometry qualitative analysis would receive high priority in treatment; this is indeed so. The chapters (2, 8 and 9) dealing with the measurement of mass and qualitative analysis, together with the relevant appendixes—No. 1 giving the masses and isotopic-abundance ratios for various combinations of carbon, hydrogen, nitrogen and oxygen, No. 2 giving nomograms for determining the origin of metastable ions, No. 5 giving possible peaks in the mass spectra of fluorocarbons and their composition, No. 6 giving the mass spectrum of Fluorolube residues (above mass 69)

and No. 7 giving some common mass doublets—occupy more than one-third of the book. However, it is valuable to have, for the first time, a full and authoritative exposition of this relatively new field of work.

All the usual features of mass-spectrometric technique and instrumentation are described in other chapters on instruments, abundance measurement, sources of positive ions, sample handling, recording of positive-ion beams and types of ions in mass spectra. All the other applications of mass spectrometry are condensed into one final chapter. In addition, two further appendixes are added: No. 3 giving a table of the masses and abundances of naturally occurring isotopes and No. 4 giving international atomic weights. Finally, some 2200 references are listed.

To deal with all this work has clearly demanded immense effort from the author. Inevitably, the quality of presentation in all these fields is bound to be somewhat variable, since no one person could claim to have special knowledge in all aspects of the subject. In fact, with a subject growing so rapidly, one wonders at what point in time an all-embracing book ceases to be a worth-while exercise. It is apparent that, even in this book, some decisions have had to be taken as to what to include and what to exclude. For example, on pp. 11 to 15, dealing with the now common double-focusing instruments, in which simultaneous correction is made for direction and energy inhomogeneities, the basic equations of Herzog and of Mattauch and Herzog have been omitted. This was probably a wise decision, but it does mean the reader cannot so readily grasp that a series of data may be chosen at will and that better combinations of electric and magnetic fields may yet be devised. Again, on p. 98, where the powerful isotope-dilution technique for determining trace elements is described, the stages involved in the derivation of equation (32) are omitted. This is a pity, because I think that the newcomer would gain much from their inclusion, particularly with regard to insight into and appreciation of the method. It becomes easier in this way to realise that many of the elements can be detected with as little as 10^{-12} g of material present. As a final example, one may refer to quantitative analysis of multi-component mixtures in the petroleum industry (pp. 424–432). Here, only brief reference is made to the computation problems involved in dealing with the available linear simultaneous equations derived from a comparison of the mixture spectrum with each pure-component spectrum. I would have thought that this work is sufficiently extensive to have merited a much fuller treatment. However, although a number of examples of this kind can be chosen, it is clear that the author had to set a limit to the size of his book, and the main text already covers some 480 pages.

The book is excellently produced, and hardly any errors have been noticed at a first reading. It will prove to be a valuable addition to the rapidly growing library of books on mass spectrometry for two main reasons: (*a*) for the work on qualitative analysis and (*b*) as a source of reference for all the other aspects of mass spectrometry. A price of 120s. for a work of this nature is not surprising in view of the existing costs of publication, but it tends to place the volume in the luxury class for the junior research worker. This is regrettable, for there is a great deal of value here for all workers in this field.

G. P. BARNARD

X-RAY MICROSCOPY. By V. E. COSSLETT, M.A., Ph.D., and W. C. NIXON, M.A., Ph.D. Pp. xiv + 406. London: Cambridge University Press. 1960. Price 80s.

An examination of the contents of this volume shows that the authors have used the term X-ray microscopy to cover a number of distinct techniques in the fields of radiography, crystallography and X-ray emission spectroscopy. The several properties of X-rays rendering them of special value in micro-scale investigations are their short wavelength, their great power of penetration into matter, the simple spectral character of X-ray emission and the rapid variation in absorption coefficient with the atomic number of the absorbing element. The various methods described include contact micro-radiography, in which an X-ray negative on fine-grain emulsion is enlarged by optical microscopy, point-projection X-ray microscopy and reflection X-ray microscopy with either mirror systems or curved crystals. A second group of techniques comprises X-ray absorption micro-analysis, X-ray emission micro-analysis and X-ray micro-diffraction, and it is with the applications of these methods that the analyst will be principally concerned.

It is less than ten years ago that Castaing first investigated the use of a fine beam of electrons to bring about direct excitation of X-rays in micro regions of a specimen, with consequent information about the distribution of elements therein. Cosslett and his colleagues have extended this idea to a scanning system in which a television technique is used to display a picture of the specimen in terms of variation in characteristic X-ray emission. By this means the distribution

of a particular element is made directly visible down to below the micron scale. Already there are available several commercial versions of these "electron probe analysers," and their potential value in metallurgical analysis cannot be over-emphasised. X-ray absorption micro-analysis is another exciting development that has been used with significant results in the biological field.

Cosslett and Nixon have arranged their account so as to provide detailed treatment of the several aspects of X-ray microscopy, covering in each instance the general principles, practical details of their use and examples of applications. The book is comprehensive, authoritative and readable, is provided with a good bibliography and contains thirty-two plates, with excellent reproductions of representative X-ray micrographs. It will undoubtedly qualify as the standard text on a subject to which the authors, in spite of a modest disclaimer in their preface, have made such notable contributions.

B. S. COOPER

ANALYTICAL CHEMISTRY OF THE RARE EARTHS. By R. C. VICKERY. Pp. viii + 139. Oxford, London, New York and Paris: Pergamon Press. 1961. Price 40s.

Information on the analytical chemistry of the rare earths is widespread, like the rare earths themselves, but the author has gathered together reliable analytical information on this subject and presented it, without unnecessary detail, in a clear and concise way. With such an extensive subject it would be unreasonable to expect to find answers to all related problems in a book of this size, but emphasis has been placed where it is most needed.

The author, who is an expert in this field, has obviously given considerable forethought to lay-out, referring first to the historical aspect of the subject then to gravimetric, volumetric, spectrophotometric, spectrographic, X-ray, polarographic and solvent-extraction methods; for good measure a chapter is devoted to radiochemical techniques.

On page 11, the reference to pentavalent chromium should read sexavalent chromium, but, with this exception, no errors are apparent, although the values given for the accuracies of the polarographic procedures on pages 132 and 133 lose their significance in the absence of any indication of the levels of europium and ytterbium involved, unless the author is really referring to "sensitivities." In future editions the author should include typical compositions; information that could conveniently be included in Table 2—1.

With a book like this available, no analyst having an interest in these fascinating and challenging Group III (sub-group A) elements can claim to be up-to-date unless this publication is at his right hand.

W. T. ELWELL

ORGANIC ELECTRONIC SPECTRAL DATA. Volume I, 1946–1952. Edited by MORTIMER J. KAMLET. Pp. xiv. + 1208. New York and London: Interscience Publishers Inc. 1960. Price (single volume) 215s.; (subscribers to whole series) 188s. Volume II, 1953–1955. Edited by HERBERT E. UNGNADE. Pp. x + 919. Price (single volume) 132s.; subscribers to whole series) 113s.

This venture aims at providing a comprehensive catalogue of ultra-violet spectral data for organic compounds. Volume III will cover 1956–1957, Volume IV 1958–1959 and Volume V 1960–1961. The first two volumes contain the work of fifty abstractors who went through 10,000 papers in seventy journals and reported 45,000 sets of data. The project was aided financially by various interests, and the publications, although expensive, are evidently subsidised.

The arrangement of compounds is according to molecular formulae; the symbols are in alphabetical order, *e.g.*, $C_9H_{12}N_2O_4S$, and the compound name is given below the formula. When possible, the solvent used is stated, and the data are given as $\lambda_{max.}$ in millimicrons, with $\log_{10}$ of the molecular extinction coefficient ($\log \epsilon$) in brackets, stated to 0·01 logarithmic unit if the data justify it. Many papers contain absorption curves without numerical data, and in these instances the abstractors have estimated the values of $\lambda_{max.}$ and $\log\epsilon$ and the figures given are underlined. When the published absorption curves exhibit fine structure, only the main maximum or the centre of the fine-structure system is entered and is labelled with the letter f after the wavelength. The final column lists the code number of the journal, the page number of the paper and the last two ciphers of the year in which it was published. The complete reference is obtained by turning to the section at the end of the book that lists all articles abstracted. The journals are arranged

in order of their code numbers, the articles in order of volume, year and page. "Errors" have been dealt with by correcting matters of nomenclature, but those apparently depending on techniques or unknown chemical or photochemical processes are reported faithfully.

The literature of the period before the second World War was in the main based on *photographic* spectrophotometry, and the task of tracing studies on particular compounds is not an impossible one in a good library. There is a good index for 1930–1954, which gives references only (Ultraviolet and visible absorption spectra, H. M. Hershenson, Academic Press, 1956). *Photoelectric* spectrophotometry became widespread just after the War, and the editors are fully justified in beginning at 1946, although it is by no means safe for research workers to neglect the older literature.

The plan of the present work is eminently sound, and the abstracting and checking have obviously been done well. In testing the coverage, I looked for a paper on pyridoxin and related compounds read before an International Congress on Analytical Chemistry (Lunn, A. K., and Morton, R. A., *Analyst*, 1952, **77**, 718), which I thought contained useful data. For the editors, it is sheer bad luck that *The Analyst* is not on their list of journals abstracted and that I should be the reviewer!

Combining truth and kindness, it is safe to predict that these reference books will be heavily used and that in most research laboratories they will soon pay for themselves in saving unnecessary labour. They are to be welcomed warmly.

R. A. MORTON

SYNTHESIS AND ORGANISATION IN THE BACTERIAL CELL. By E. F. GALE. Pp. viii + 110. London and New York. John Wiley & Sons Ltd. 1960. Price 28s.; $3.50.

Like so many current books on biochemistry, and doubtless on other subjects as well, this one depends on the activities of analysts to a far greater extent than their interest in its subject matter might indicate. For without the application of pretty difficult analytical procedures—many of which might equally well be described as difficult pretty analytical procedures—most of the results described by Dr. Gale in this concise, but informative, little monograph could not have been got. Without the results there could have been no interpretation, and without the interpretation none of the three CIBA Lectures at Rutgers (The State University of New Jersey) forming the substance of this book would or indeed could have been given.

In chapter I, on Structure and Organisation in the Bacterial Cell, and in chapters II and III, on Amino Acid Incorporation and on Nucleic Acid and Protein Synthesis, respectively, everything, or almost everything, depends directly or indirectly on a mass of analytical results got by workers who, mainly for reasons of space, maybe, are not mentioned and may indeed be in danger of oblivion.

For this Dr. Gale is himself in no way to blame; he had, in all conscience, already overmuch to crowd into his three lecture - chapters. It seems to me, all the same, that analysts might be more encouraged to interest themselves in the sometimes apparently remote results and conclusions that have arisen from their arduous laboratory toils if those who almost unconsciously make use of the analysts' methods would a little more often and a little more openly give credit to them when it is due.

A practical advantage would also be gained from closer understanding between analyst on the one hand and biochemist or microbiologist on the other. The latter might then enquire a little more closely into the validity of the methods that have given him his results; the former might be more inclined to look critically at the methods he is proposing to use.

A. L. BACHARACH

ANALYSE QUALITATIVE RAPIDE DES CATIONS ET DES ANIONS. By G. CHARLOT. Third Edition. Pp. xiv + 82. Paris: Dunod. 1961. Price 9.50 NF.

The industrial chemist asked to review a book on qualitative analysis approaches his task with diffidence. Books on this subject are almost always written for colleges, and this is no exception. It is an introduction to the system of identifying inorganic substances by spot tests. The classical "group system" of qualitative analysis remained almost unchanged for a long time;

given a large enough sample and the requisite skill, it could yield satisfactory results. Admittedly, the elements covered were rather arbitrarily selected; even so, a competent analyst with a reasonable knowledge of inorganic chemistry in its more factual aspects could cope with most of the samples presented to him.

To-day a totally different system is emerging. Based on the kind of spot test popularised by Feigl, qualitative analysis, always on a semi-micro or micro scale, becomes a series of tests for individual elements or small groups of elements, and separations are avoided. It is a variant of this scheme that is advocated by Professor Charlot, and the fact that his booklet has now passed through three editions indicates the interest it has aroused. Charlot's scheme is not intended to deal with traces; it is a system for identifying any of a series of elements or ions present to more than 0·1 per cent. of the whole, the size of sample being, perhaps, 50 mg, and, as presented in this booklet, it covers rather more than sixty inorganic ions. The instructions are generally clear, although there are occasional signs of hasty writing or revision: for example, on p. 60, the "Remarks" under the heading of reactions with barium belong really to the paragraph above, dealing with silver nitrate. This will not worry a teacher, but might confuse a student.

This is a teaching manual and not a handbook for the professional laboratory, but it deserves to be read by both lecturers and professional analysts. Qualitative inorganic analysis has acquired a reputation as a dull mechanical subject, mostly because it was taught in a dull mechanical way. There is nothing dull or mechanical in the school of analytical chemistry at the École Supérieure de Physique et de Chimie Industrielles; on the contrary, its ideas are stimulating, and serious teachers of analytical chemistry cannot fail to be stimulated by reading them. They may strongly disagree with these ideas, but what matters is that they give fresh thought to the contents of their courses. I do not know how qualitative analysis should be taught, nor how much should be taught, but I do regard it as rather important. In a large establishment well equipped with physical apparatus, it may not often be necessary, but there are few laboratories, large or small, where from time to time a knowledge of this art is not essential. To an outsider it appears that, within academic walls, analytical chemistry is fighting a losing battle, and it certainly will not regain any lost ground with weapons designed—although no doubt refurbished here and there in the interval—a hundred years ago.

In the industrial laboratory, although the question "What is it?" is sometimes asked, more often one hears "Does it contain x?" or "Is it y or is it z?" Here, well designed spot tests are more useful than the traditional scheme, and Charlot's text-book is an excellent introduction; if the analyst wishes to cover every possibility he will need a much fuller treatment.

H. N. Wilson

Advances in Analytical Chemistry and Instrumentation. Volume I. Edited by Charles N. Reilley. Pp. viii + 445. New York and London: Interscience Publishers Inc. 1960. Price $12.00; 90s.

In this new venture an attempt is being made to bring new analytical developments into working practice and, moreover, to discuss critically, from time to time, conventional classical procedures in analytical chemistry. It is suggested that the volumes will appear annually, and the first volume certainly caters for the most diverse tastes.

There are chapters on tetraphenylboron and thioacetamide as analytical reagents, and new ideas are presented on organic micro-analysis and on the determination of fluorine. Gas-chromatographic detectors, near-infra-red spectrophotometry and theories of electrode processes all find a place, and the individual articles are written by experts in the form of critical reviews.

There is no doubt that this innovation will appeal to those analytical chemists who, while ordinarily engaged in specialised activities, are sufficiently wide-awake to want to keep abreast of new developments and wish, moreover, to do so by reading articles that present the essential ideas in small compass.

J. Haslam

Publications Received

GAS CHROMATOGRAPHY. By ERNST BAYER. Pp. xii + 240. Amsterdam, London, New York and Princeton: Elsevier Publishing Company; London: D. Van Nostrand Company Ltd. 1961. Price 25s.

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